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13. ABSTRACT (cont from p.1) dimethylcuprate as the nucleophile, conjugate addition at the interface of the carbon-carbon double bond with the bicyclic system was attempted. It was initially predicted that the methyl group would add to the number 4 position, creating both a quaternary carbon and a stereocenter. In actuality, the cuprate reagent added to the competing electrophilic carbonyl of the lactone group on the opposite ring with subsequent ring opening upon acidic work-up conditions. Continuing, tertiary stereocenters were investigated via two reactions. The first, known as catalytic hydrogenation, consisted of the reaction of hydrogen gas with the α,β-unsaturated ester mediated by the surface properties of several metallic catalysts. Spectroscopic analysis confirmed successful synthesis of a single tertiary stereocenter isomer. The second reaction employed Grignard reagents to add directly to the ketone carbonyl of the fused bicyclopentane. In this case, the sterics of the convex side of the molecule caused the Grignard reagents to act as bases, extracting the hydrogen bonded between the carbonyls. Lastly, secondary stereocenters were created through the use of hindered reduction reagents, such as K-selectride, attacking the ketone carbonyl of the fused bicyclopentane. The structural and stereochemical outcomes of the reactions have been determined through spectroscopic methods. Primarily, Nuclear Magnetic Resonance (NMR) and Gas Chromatography/Mass Spectrometry (GC/MS) have allowed conclusive confirmation of structure and reaction products. Ultimately, the results of this work will constitute a significant contribution to synthetic methodology, particularly as it pertains to terpene synthesis.

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ABSTRACT:

Organisms in nature are often capable of producing complicated molecules that are difficult to synthesize in the laboratory. Some of these compounds demonstrate substantial biological activity and are useful in the treatment of disease as pharmaceuticals. One such class of compounds, dolabellanes, has demonstrated significant anti-tumor capability but is found principally in marine species inhabiting sensitive ecosystems. Thus, interest in their production through organic synthesis methodology is high. These compounds are also synthetically challenging targets, containing a multi-functionalized eleven-membered ring fused to a cyclopentane ring bearing three stereocenters. This project has been aimed at investigating means of introducing various types of stereocenters relevant to dolabellanes in an effort to simplify their ultimate synthesis.

To overcome the stereocontrol problems involved in reactions of conformationally labile cyclopentane rings, this project has postulated that employing a fused bicyclopentane system can permit the creation of stereocenters with predictable chirality. This is made possible by the rigidity of the fused system, which is energetically constricted to one conformation. In this project, the ketone in the previously prepared molecule was converted into an alkene conjugated with a carbonyl-containing ethyl ester. Two reaction methods were studied to prepare this α,β-unsaturated ester. The Horner-Wadsworth-Emmons reaction was employed using triethyl phosphonoacetate as the reagent to introduce the conjugated ethyl ester. Secondly, the Wittig reaction was then explored to introduce the same carbon-carbon double bond motif using an ylide reagent. The latter reaction proved to be most successful and was used exclusively in subsequent reactions.

The creation of a quaternary stereocenter was first explored. Using lithium dimethylcuprate as the nucleophile, conjugate addition at the interface of the carbon-carbon double bond with the bicyclic system was attempted. It was initially predicted that the methyl group would add to the number 4 position, creating both a quaternary carbon and a stereocenter. In actuality, the cuprate reagent added to the competing electrophilic carbonyl of the lactone group on the opposite ring with subsequent ring opening upon acidic work-up conditions. Continuing, tertiary stereocenters were investigated via two reactions. The first, known as catalytic hydrogenation, consisted of the reaction of hydrogen gas with the α,β-unsaturated ester mediated by the surface properties of several metallic catalysts. Spectroscopic analysis confirmed successful synthesis of a single tertiary stereocenter isomer. The second reaction employed Grignard reagents to add directly to the ketone carbonyl of the fused bicyclopentane. In this case, the sterics of the convex side of the molecule caused the Grignard reagents to act as bases, extracting the hydrogen bonded between the carbonyls. Lastly, secondary stereocenters were created through the use of hindered reduction reagents, such as K-selectride, attacking the ketone carbonyl of the fused bicyclopentane.

The structural and stereochemical outcomes of the reactions have been determined through spectroscopic methods. Primarily, Nuclear Magnetic Resonance (NMR) and Gas Chromatography/Mass Spectrometry (GC/MS) have allowed conclusive confirmation of structure and reaction products. Ultimately, the results of this work will constitute a significant contribution to synthetic methodology, particularly as it pertains to terpene synthesis.

Keywords: dolabellane, terpene, stereocontrol, reduction, hydrogenation

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INTRODUCTION:

In this project, the synthetic obstacles presented by a terpenoid class of compounds called dolabellanes will be addressed. Dolabellanes diterpenoids have been isolated from both marine and terrestrial sources.[1] In aquatic environs, sea mollusks, soft corals, and brown algae contain various forms of the compounds. Fungi, liverwort, and even higher plant species produce them on land.[3] This natural ubiquity is a testament to the plethora of biological activities associated with dolabellanes. In fact, dolabellanes have recently attracted attention due to their pharmacologically pertinent effects. An impressive array of medicinal applications has been demonstrated, including anti-cancer, antibacterial, antifungal, antimalarial, and antiviral applications. A specific example consists of dolabellane extracts collected from the Formosan soft coral *Clavularia inflata* var. *luzoniana* using methylene chloride as the solvent. These extracts showed substantial cytotoxicity to human colon adenocarcinoma and mouse lymphocytic leukemia.[2]

In some cases, the natural availability of complex organic molecules is limited. The compounds might be produced by organisms living in fragile, protected environments. For example, dolabellanes were originally discovered in marine organisms.[3] Disruption of these ecological locations, like coral reefs, in the name of chemical extraction is generally unacceptable. Moreover, the physical amount of the compounds in the organisms is often small. It is also especially difficult to extract specific organic compounds from higher-order organisms, such as animals. Therefore, methods for synthesizing usable quantities of the compounds must be pioneered.

Organic synthesis principally concerns the production of complex molecules from simple base compounds. Generally, a series of linear steps are postulated, whereby the product of one

reaction becomes the starting reagent for the next. Often, chemists find it prudent to begin the thought process with the target molecule and work backwards until a commercially available starting compound is identified. This technique is known as retrosynthetic analysis.[4] Synthetic methodology, another component of synthesis research, looks at ways to prepare a functional group or a structural feature desirable as an archetype for families of compounds. Methods for introducing these particular elements are explored in order to evaluate their relative effectiveness. As such, synthesis procedures seek to optimize the results of reaction sequences, making many sought-after molecules accessible. Overall, for both aspects of synthesis, the goal is to solve synthetic challenges by simultaneously minimizing steps and maximizing yield.

Dolabellanes are complex structures that essentially present two synthetic hurdles. The first is the incorporation of a large, eleven-membered ring structure; the second is the presence of various stereocenters. Four terpenoids are highlighted in **Figure 1.** The first two compounds are dolabellanes and contain quaternary stereocenters, the most difficult to synthesize. The third compound in the center of the figure contains a tertiary stereocenter while the fourth compound at the bottom contains a secondary stereocenter. Each of these latter two could potentially be epimerized with the long carbon chain closed to form dolabellanes. However, this could only happen after successfully introducing the stereocenters labeled in **Figure 1.**

Because all three aforementioned stereocenters have relevance to the dolabellane compounds of long-term interest, this project has sought to investigate each one. The retrosynthetic analysis of a dolabellane is depicted in **Figure 2** and shows the logical progression of the dolabellane diterpene to a rigid bicyclic precursor (bicyclo-[3.3.0]-octane system). It is this precursor around which the project has revolved. This molecule has allowed for the number of synthetic steps to reach fixed stereocenters to be reduced to two. Primarily, this has been due

to the rigidity of the molecule and its tendency to maintain a single conformation in three-dimensional space.

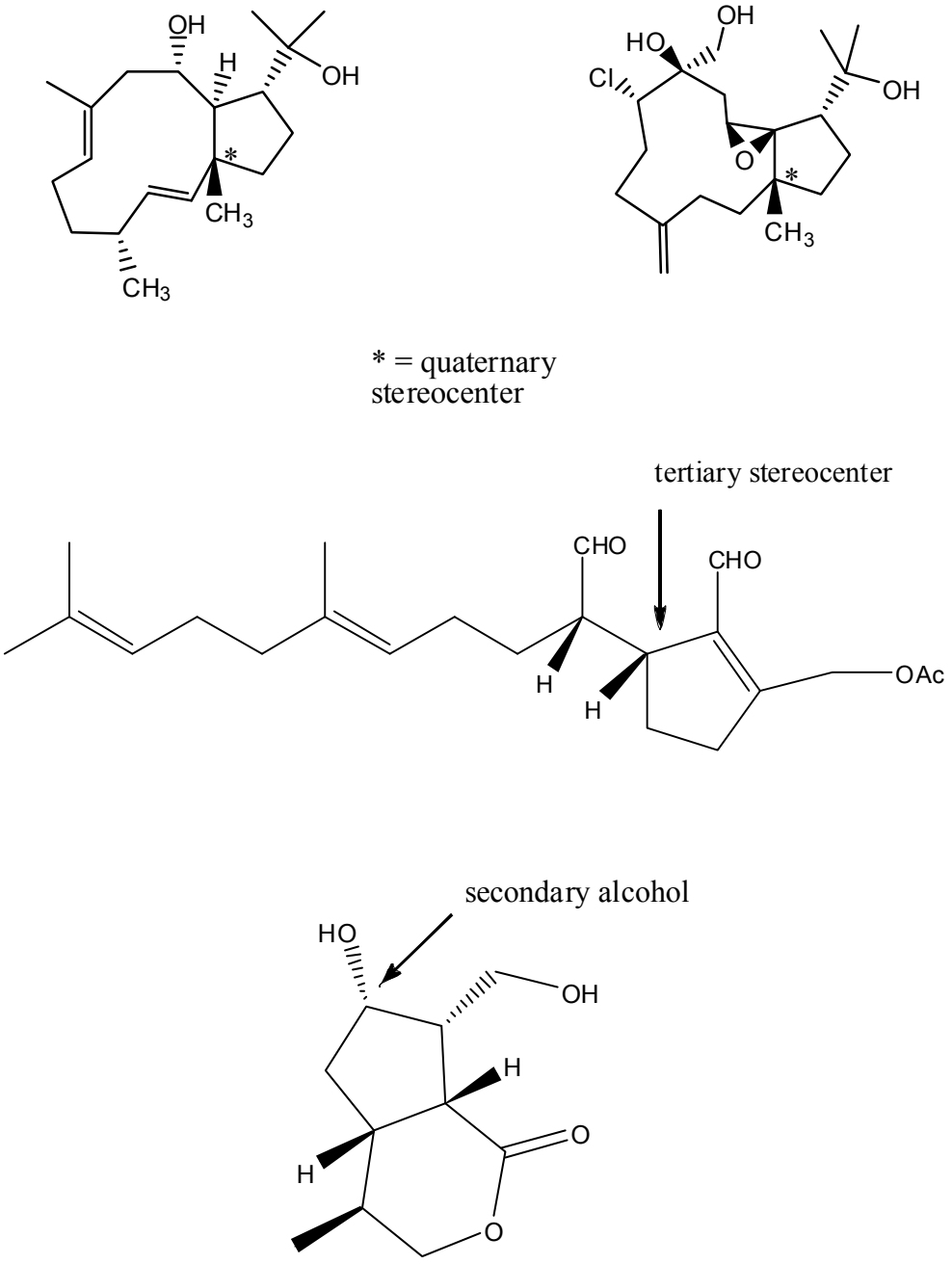


Figure 1: Examples of Terpenes with Various Stereocenters

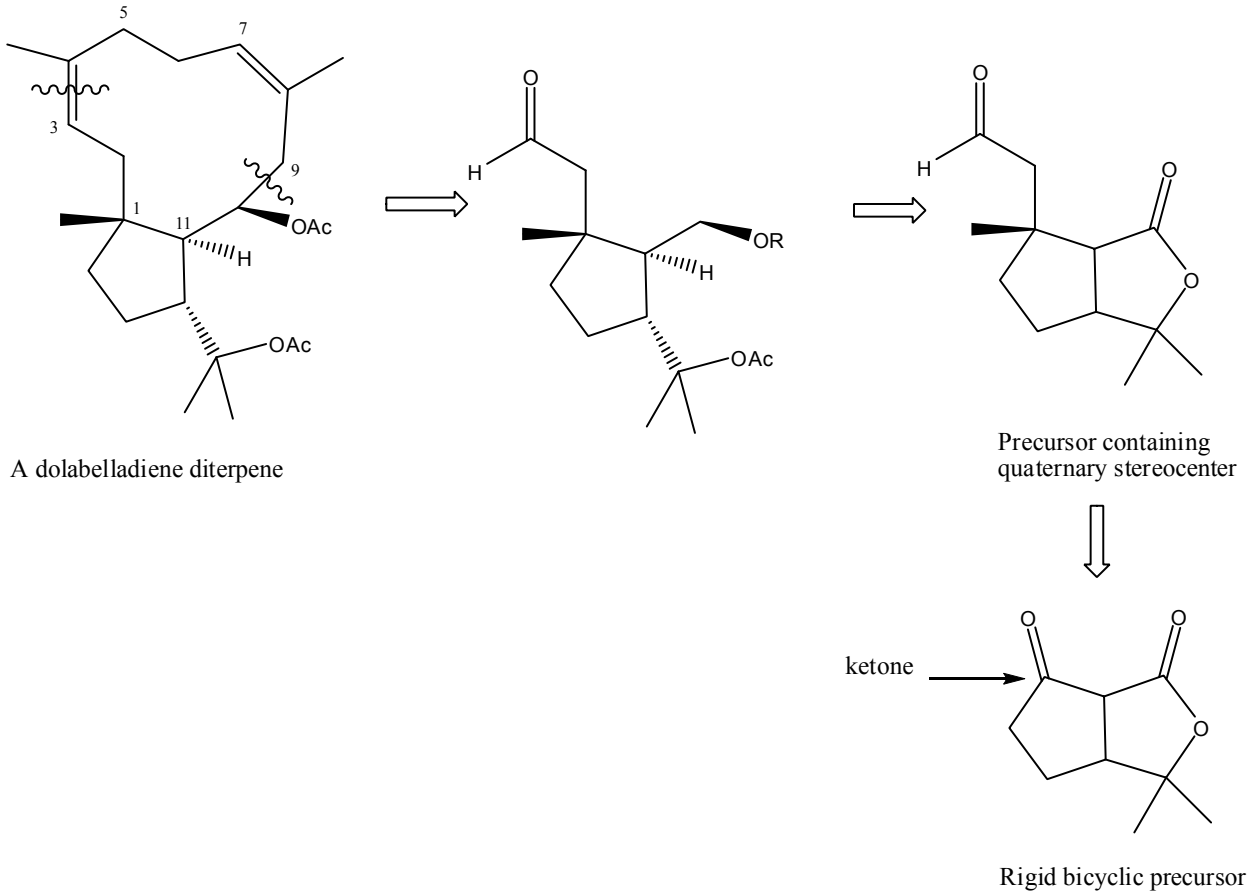


Figure 2: Retrosynthetic Analysis of a Dolabellane Diterpene

One of the keys to transforming this rigid bicyclic precursor, to dolabellane or other terpenes, is the stereochemically controlled modification of the ketone carbon. An explanation of stereocenters is provided in **Appendix B**, along with a presentation of their biological relevance. The conversion of the ketone to a quaternary stereocenter was the primary focus of this project until late January of 2008. The remainder of the project dealt with the synthesis of secondary and tertiary stereocenters at the location of interest that pertain to dolabellanes. This proposal examined using the rigidity of the fused bicyclic template to introduce stereocenters at

this position in a synthetically efficient manner. This has proven successful through both hydride reduction and catalytic hydrogenation reactions. A representative overall synthetic scheme is presented in **Figure 3.** Previous precedents have not accomplished similar syntheses in fewer than five or six reaction steps. The success in this project has thus improved yields and made large-scale syntheses more practical. In addition, the bicyclic system provides a logical structure for subsequent introduction of the eleven-membered ring. Success in this project has thus contributed to expanded dolabellane study.

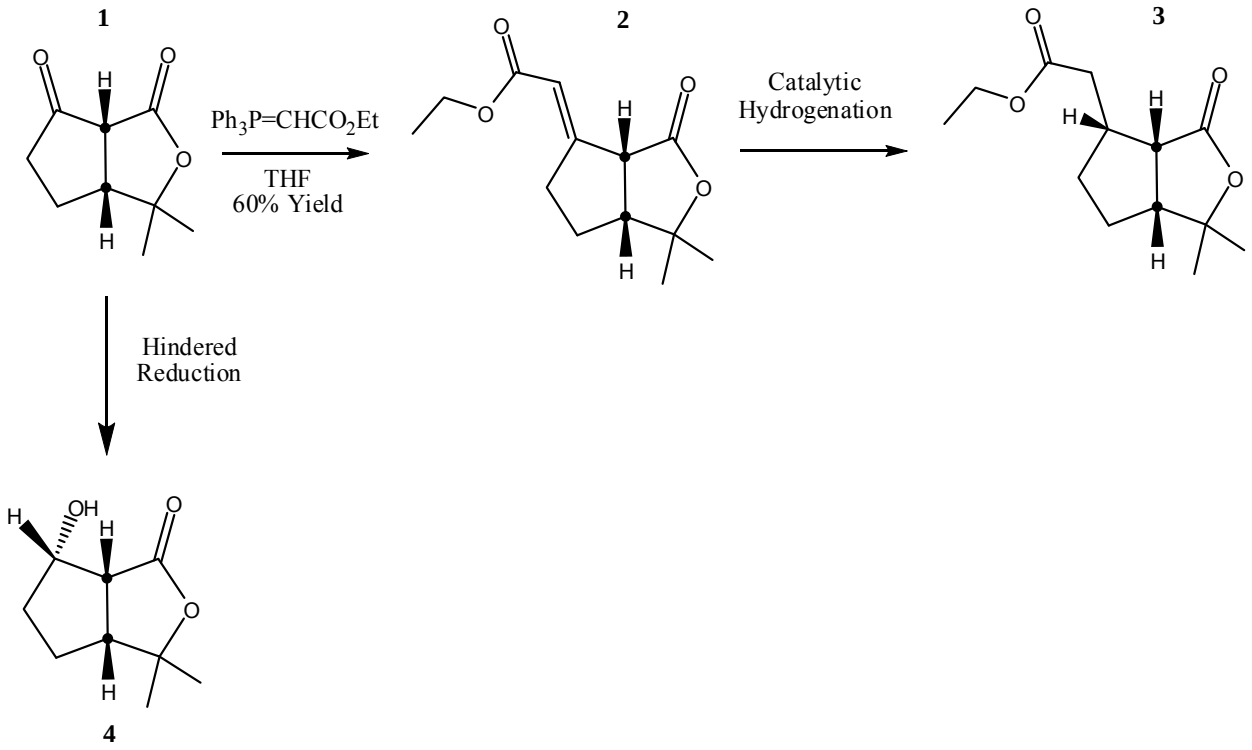


Figure 3: Overall Synthetic Scheme

RESULTS AND DISCUSSION:

Goal I:

The first stage of the research sought to convert the ketone group on the left side of the fused bicyclic compound to an alkene. Two means of accomplishing the conversion to a carbon-carbon double bond are known and both were examined. The resulting system is termed conjugated because there are two double bonds with a single bond separating them. This double bond-single bond-double bond motif has interesting electronic implications that were hypothesized to allow for the introduction of the quaternary stereocenter in the second synthetic step.

The first method for preparing this alkene was via the Horner-Wadsworth-Emmons reaction. In this case, phosphonate ester reagents were employed as the carbanions, which is the term used for the carbon molecules that are added to the system.[5] Specifically, triethyl phosphonoacetate (**A**) was the phosphonate ester of choice. Commercially available, this ester was relatively simple to work with and allowed for the introduction of a conjugated α, β-unsaturated system as shown in **Figure 4**. The reaction yielded two isomeric forms based on different confirmation around the double bond. Both forms were useful and later reacted equally to form the same stereocenters.

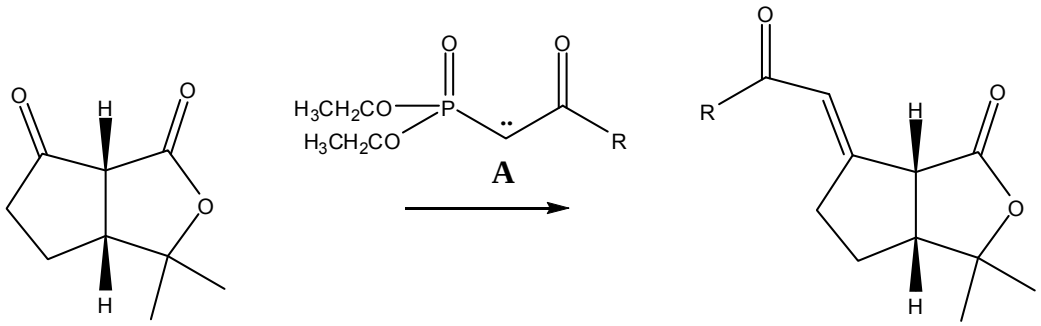
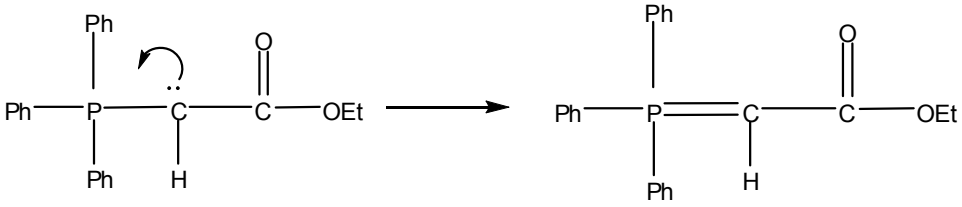


Figure 4: Horner-Wadsworth-Emmons Reaction

In order for the reagent (**A**) to be modified to its active deprotonated form, the phosphonate ester had to be reacted with a strong base to remove the hydrogen from the carbon of interest, creating the negatively charged carbanion. The base of choice was potassium *tert*-butoxide. Once deprotonated, the reagent was then reacted with the bicyclic starting compound to affect the desired alkene creation. Details of the mechanism, including electron transfers, can be referenced in **Appendix C**.

The second means of introducing the alkene is via the Wittig reaction. Here, the carbanion reagent is a triphenyl phosphonium ylide (**B**), which can be seen in **Figure 5**. This reagent is unique in that the negative charge on the carbon is stabilized both by positive charge on the phosphorus atom and a resonance structure with a double bond to the phosphorus. With the specific reagent studied, the same conjugated product as the Horner-Wadsworth-Emmons reaction was created.



B

Figure 5: Triphenyl Phosphonium Ylide Reagent

Like their phosphonate ester cousins, ylides must be deprotonated in order to become an active nucleophile. The strong base of choice initially was n-butyl lithium, which is available as a stable organometallic solution. After experiencing difficulty converting the Wittig reagent to the ylide from its commercially available form, an alternative procedure was undertaken for preparing the active agent, **2**. The solid salt was dissolved in a water and toluene mixture. Then, sodium hydroxide was added to accomplish two chemical objectives. As a base, sodium

hydroxide was able to deprotonate the reagent to the active ylide form. With the sodium cations introduced, an insoluble precipitate, sodium bromide, could form in the aqueous phase that removed the bromide anions. The ylide, given its large phenyl rings and largely organic nature, preferentially dissolved in the toluene layer. Once the aqueous phase was separated and the toluene was removed, the ylide was ready to immediately be reacted with the bicyclic ketone. Mechanistic details of the Wittig reaction can be referenced in **Appendix C**.

Initially, the Horner-Wadsworth-Emmons reaction was expected to afford better results for several reasons. The phosphonate ester reagents are more nucleophilic and more basic and thus should react more thoroughly. Moreover, the dialkylphosphate salt byproducts of this reaction were purportedly easier to separate using aqueous extraction. By contrast, the Wittig byproduct, triphenyl phosphine oxide, is notoriously difficult to remove. From literature searches, a four-to-one ratio of the carbanion to the bicyclic starting compound was expected to give optimal conversion of the ketone to the unsaturated alkene system. Nuclear Magnetic Resonance (NMR) was used to analyze the crude product mixture. A detailed discussion of NMR interpretation is found in **Appendix D**, which also includes an explanation of how the axes should be interpreted. However, as evidenced by the NMR spectrum in **Figure 6**, standard work-up procedures were unable to remove the unreacted triethyl phosphonoacetate.

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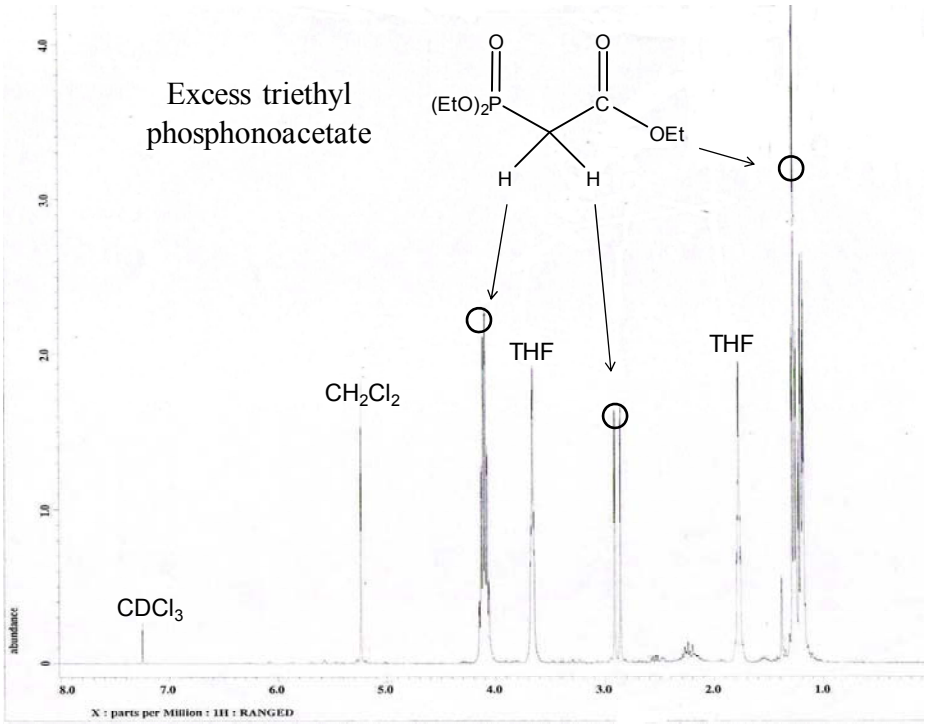

Figure 6: NMR Spectrum Highlighting Excess Phosphonoacetate in 4:1 Ratio

Reducing the ratio to 2:1 did not mitigate this effect, as highlighted by **Figure 7**, which displays an NMR spectrum again with excess triethyl phosphonoacetate. Rather than empirically investigate the perfect ratio, we concluded that we would explore the viability of the Wittig reaction.

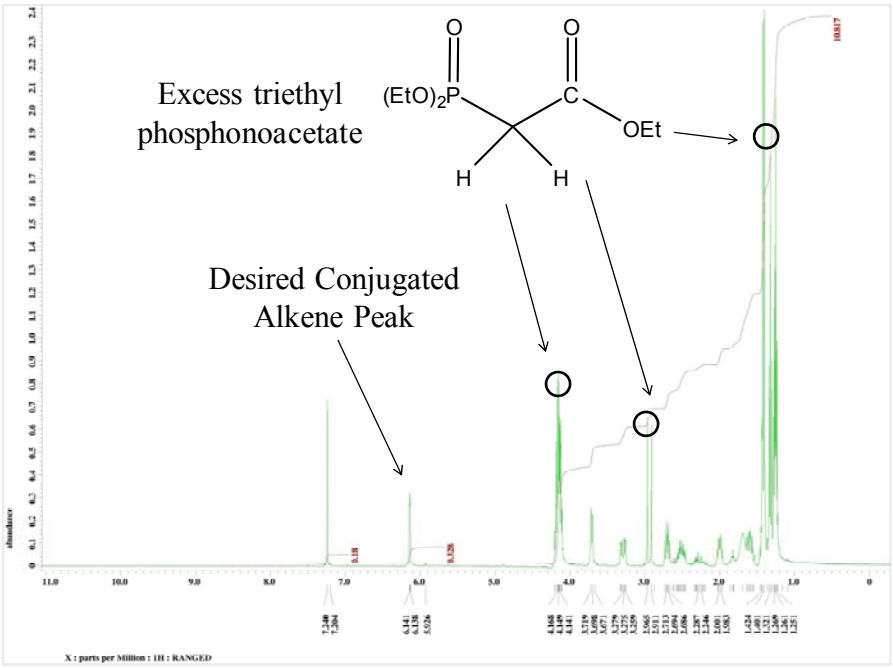

Figure 7: NMR Spectrum Highlighting Excess Phosphonoacetate in 2:1 Ratio

As mentioned, the primary difficulty in the Wittig reaction would be removing the triphenyl phosphine oxide byproduct, which can be seen in **Figure 36** in **Appendix C**. Flash chromatography proved to be the experimental solution to this problem, as the phosphine oxide stuck firmly to the polar silica of the column. Chromatography is discussed in **Appendix E**. From thin layer chromatography (TLC), it was known that a 3:2 ratio of ethyl acetate to hexane caused the desired product to migrate with a retention factor of approximately 0.50. A rule of thumb is that in moving to an actual column chromatography, one should transition to a solvent mixture which results in retention factors of about 0.35. Thus, after investigating, we determined that an 8:1 mixture of diethyl ether to ethyl acetate yielded the conditions that could effectively leave the phosphine oxide on the column while aliquots of the pure desired compound could be collected in fractions. The purity of the desired alkene was confirmed by the presence of a

singlet, labeled **b**, at approximately 6.1 ppm, indicative of the hydrogen of the dominant isomer. The absence of the minor isomer was confirmed by the lack of the characteristic singlet at about 5.9 ppm. Both of these features are highlighted in **Figure 8,** an NMR spectrum of pure unsaturated ester, which will henceforth be referred to as **compound 2**. Although the major isomer was the predominant product and was the isomer collected, presence of the minor isomer in the product mixture was of no concern, given their equal reactivity, as previously stated. Combining the desired fractions, evaporation of the solvent afforded the pure compound in an approximately sixty percent yield.

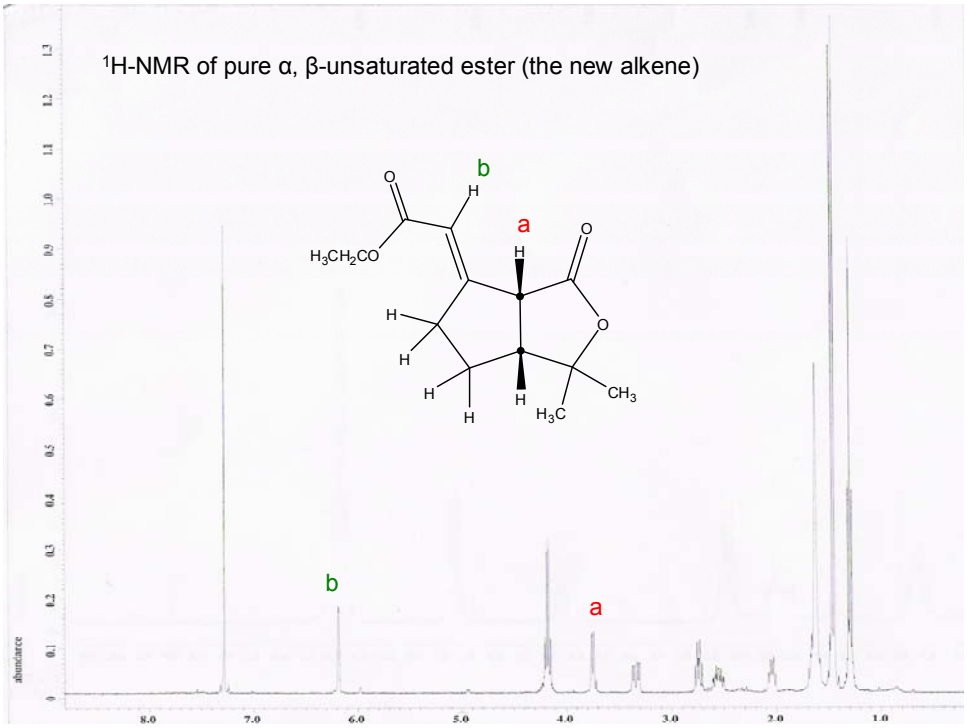

Figure 8: NMR Spectrum of Pure Unsaturated Ester, Compound 2

Goal II – Introduction of a Quaternary Stereocenter

From September until January, a second synthetic step that sought to transform the carbon-carbon double bond into a single bond with the insertion of an alkyl group at the carbon fused within the ring structure was explored. To accomplish this addition of a quaternary stereocenter, the conjugate addition reaction was explored. According to this accepted mechanism, the number four carbon of a conjugated system serves as the electrophilic center to be attacked by a nucleophilic carbon reagent. The use of select organometallic reagents allows the experimenter to control addition at this location, rather than the alternative number two carbon. Organocuprate reagents have demonstrated consistent ability to react at this position when combined with α, β-unsaturated carbonyl compounds of the sort synthesized in the first synthetic pathway.[6] As will be discussed, this was not the result observed and has opened interesting possibilities for future fused bicyclic and dolabellane chemistry. Copper(I) salts combined with alkyl lithium reagents generate cuprate reagents known as homocuprates. Homocuprate reagents are the most widely used organocopper reagents and efficiently transfer one equivalent of an alkyl group to the number four position.[7] Discussion of the mechanism of 1,4-conjugate addition and nucleophilic tendencies that discriminate for this specific addition are presented in **Appendix C**.

Because of the unique reactivity and stability considerations of the reagents employed, this reaction proved to be the most difficult experimentally. This project exclusively studied methyl cuprates due both to their structural simplicity, reactivity, and their pertinence to dolabellanes. Copper(I) halides served as the means of introducing copper. When combined with methyl lithium, copper and one methyl group initially bond to form methylcopper. The reaction is proceeding correctly if a yellow color emerges, indicative of the insoluble

methylcopper polymeric precipitate in diethyl ether.[7] A colorless transition occurs once the second equivalent of methyl lithium adds to give the lithium dimethylcuprate reagent needed for the actual addition reaction. This color change again served as a visual confirmation of successful reagent preparation. The reaction sequence leading to lithium dimethylcuprate (Me_2CuLi) is shown in **Figure 9.**

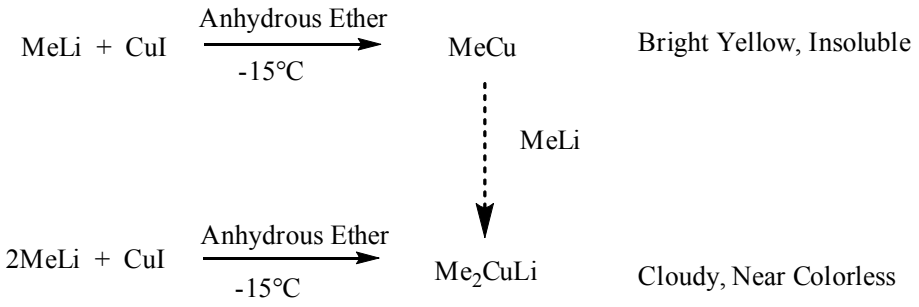

Figure 9: Synthesis of Lithium Dimethylcuprate[8]

The corresponding mechanisms pertinent to the chemistry of this project are presented in **Appendix C**.

Copper(I) iodide was the principal copper(I) salt investigated. This particular copper reagent offered the advantage of being fairly resistant to water absorption and having the most widespread literature precedence. Homocuprates prepared from copper(I) iodide reacted as predicted with the pilot reactions, as evidenced by the GC/MS Spectrum in **Figure 57** in **Appendix E**. Thus, the potential for cuprates to achieve 1, 4-conjugate addition with general α, β-unsaturated esters was confirmed. Also, valuable experience was gained in handling these oxygen, water, and temperature sensitive reagents. Several difficulties emerged when moving to the more complicated bicyclic α,β-unsaturated ester, **compound 3**. The first was the small-scale nature of the reactions themselves. Working on a 50 mg scale requires precision in all experimental parameters, some of which are outside of the control of the chemist. If air is

inadvertently admitted into the reaction mixture or the reagents are in any way contaminated, the reaction will fail. One batch of impure methyl lithium resulted in no fewer than four failed cuprate reactions. Only after accounting for every other variable was the methyl lithium deemed to be the problem. Although it seems the desired quaternary carbon proved elusive, interesting results were nonetheless obtained and are discussed following a presentation of the several variables changed in an effort to accomplish successful reaction.

Six variables were altered in efforts to begin exploring the scopes and limitations of the project and to find the most suitable reaction parameters. First, the copper(I) reagents were changed. Although copper(I) iodide seems to be the most popular copper-containing salt for producing homocuprates, copper(I) cyanide (CuCN) and copper(I) bromide-dimethyl sulfide ($CuBr•SMe_2$) demonstrated utility as organocuprate precursors.[7] Thus, the latter dimethyl sulfide complex was attempted in producing the same lithium dimethyl cuprate discussed previously. Because it more readily absorbs atmospheric water, the reagent was dried before use in a desiccator. This complex proved to be the most successful copper containing reagent in the synthesis of cuprate reagents, as it most reliably exhibited the characteristic color transition from the yellow of methyl copper to the clear solution indicative of the sought-after dimethyl cuprate

Secondly, different solvents were explored to find the best combination of both facilitating the reaction and dissolving the constituents. Ether proved to be the best solvent attempted in preparation of the cuprate reagent itself. However, when used in the reaction with the unsaturated ester, ether was ineffective in completely dissolving the conjugated alkene. It was known that the bicyclic compounds dissolve well in dichloromethane (CH_2Cl_2). Unfortunately, dichloromethane cannot be used in the actual preparation of the homocuprate reagents since alkyl lithium reagents react with CH_2Cl_2. Thus, a literature search was conducted

that resulted in a procedure to remove the ether used in preparing lithium dimethyl cuprate with a vacuum pump followed by the introduction of dichloromethane to actually dissolve the carbonyl-containing compound.[9] Due to the difficulty of switching solvent systems through vacuum, it was concluded that dissolving the copper reagents in a minimal volume of ether, followed by using dichloromethane to dissolve the unsaturated ester might improve the reaction conditions. Thus, once the cuprate was prepared, the unsaturated ester was dissolved in a minimal amount of CH_2Cl_2 and added to the cuprate via syringe injection.

The third reaction improvement technique was the activation of the ester and the trapping of the intermediate enolate anion by chlorotrimethylsilane (TMSCl). The enolate is formed during the reaction of the carbonyl compound and the cuprate reagent as shown in **Appendix C**. When TMSCl is added, the enolate is captured as trimethylsilyl enol ether. Literature precedents cite improved yields with this technique. In fact, use of TMSCl both increases the rate of the conjugate addition and increases the overall yield.[6] The effect is best when TMSCl is added just before the carbonyl and the temperature of the solution is decreased to -78°C. When the reaction is quenched with aqueous acid, the silyl enol ether intermediates are hydrolyzed with the creation of the desired addition product. Initially, ammonium chloride was attempted as the quenching agent, in accordance with literature precedent. However, as indicated by the GC/MS Spectrum in **Figure 10**, TMS intermediates remained in the solution. So, it was concluded a slightly stronger acid would be needed to both protonate the methylated product and cleave the TMS intermediates. Hydrochloric acid (2M) was the aqueous work-up selected, which allowed for the reaction products to be collected in protonated form after chromatography.

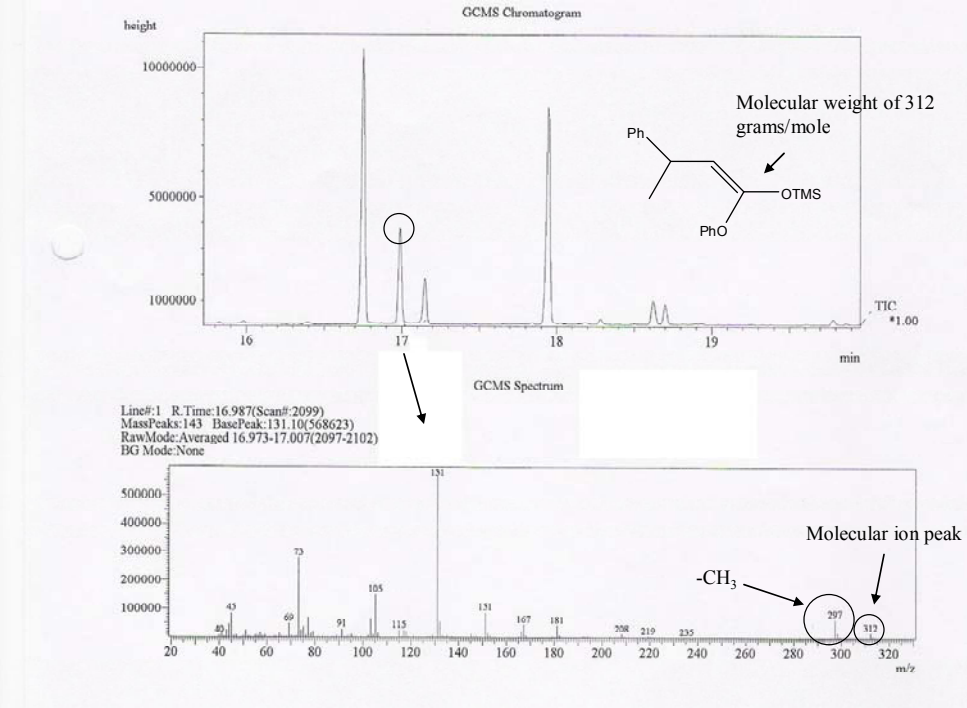

Figure 10: GC/MS of Trimethylsilyl Intermediate

The fourth variable centered on using stoichiometry to induce successful addition. Initially, the ratio of organocuprate to unsaturated ester was only slightly greater than one-to-one (1.2:1). It was reasoned that by dramatically increasing this ratio the rate of the reaction could be sped up as well. So, the ratio of lithium dimethylcuprate to α,β-unsaturated ester was increased to 5:1.

Fifth, the temperature was adjusted with the intention of finding the most appropriate level that afforded reactivity and protected the heat sensitive cuprate reagents. In most literature references, the reaction was allowed to warm slowly to room temperature (20°C) from the -78°C. This process took approximately one hour, which was followed by two hours of stirring at 20°C.[6] Interestingly, when the reaction vessel was immediately removed from the -78°C bath and allowed to warm to room temperature, the predominant isomer reacted nearly completely,

whereas the minor isomer remained. Because starting material was occasionally left over after replicating these conditions, it was reasoned the cuprate might be breaking down when the temperature climbed over 0°C before the reaction had time to go to completion. So, the reaction was maintained at -78°C for approximately 45 minutes followed by warming to -15°C and allowing to stir for another 2 hours. At this point, the reaction was quenched with 2M HCl until acidic. However, as demonstrated by the GC/MS Spectrum in **Figure 11**, the presence of unreacted starting material indicates the reaction needed to be run at a higher temperature to drive reaction of the cuprate. Finding a temperature balance was a difficult process because the structural nature of the cuprate reagents causes them to be unstable at temperatures above 0°C yet running the reaction at too low a temperature restricts any reactivity. Thus, after several iterations, it was deemed that the optimal temperature adjustment consisted of formation of the cuprate at -78°C followed by shifting to -15°C for 30 minutes when the unsaturated carbonyl was added and then to 0°C to allow for the reaction of the cuprate with the conjugated system.

Finally, conditions for quenching the reaction were studied. The purpose of the quenching step is to remove unreacted cuprate agents and to convert the enol ether to the ester.

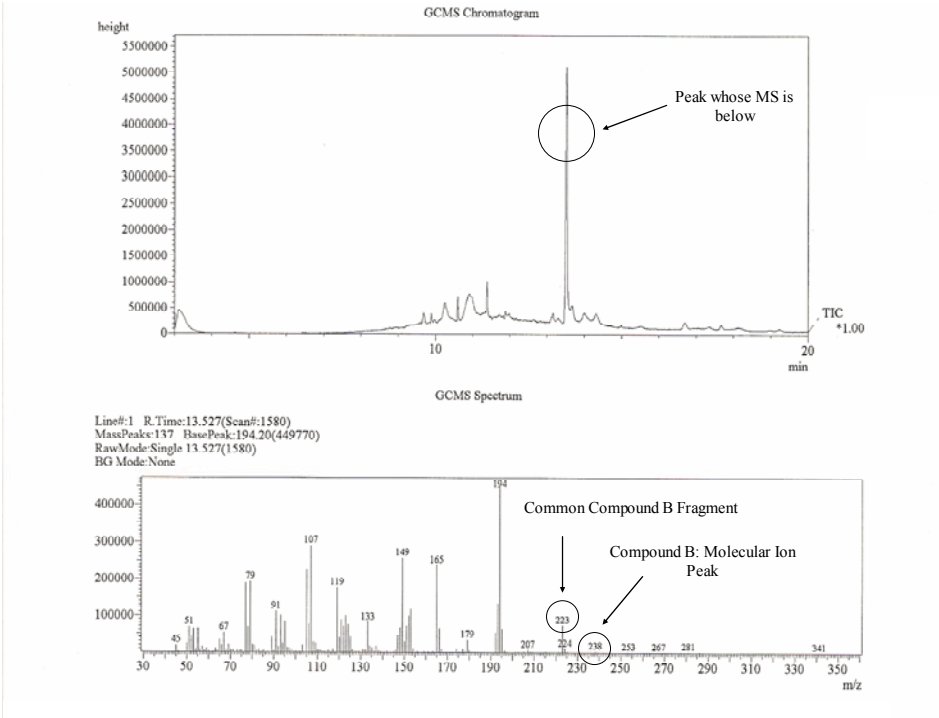

Figure 11: GC/MS Display of Unreacted Compound B

The actual results of the cuprate reaction will now be discussed and explained based on interpretation of NMR spectra collected. The NMR spectrum in **Figure 12** shows the proton spectrum of a crude post-cuprate reaction mixture. Here, it appears the predominant α,β-unsaturated alkene isomer reacts almost completely with the cuprate reagent while the other isomer does not react. A new methyl peak at the expected chemical shift even emerges, which was one the characteristic indicators of successful conjugate addition. This result seemed to indicate that, as far as the fused bicyclic system is concerned, the reaction might be occurring at the major isomer.

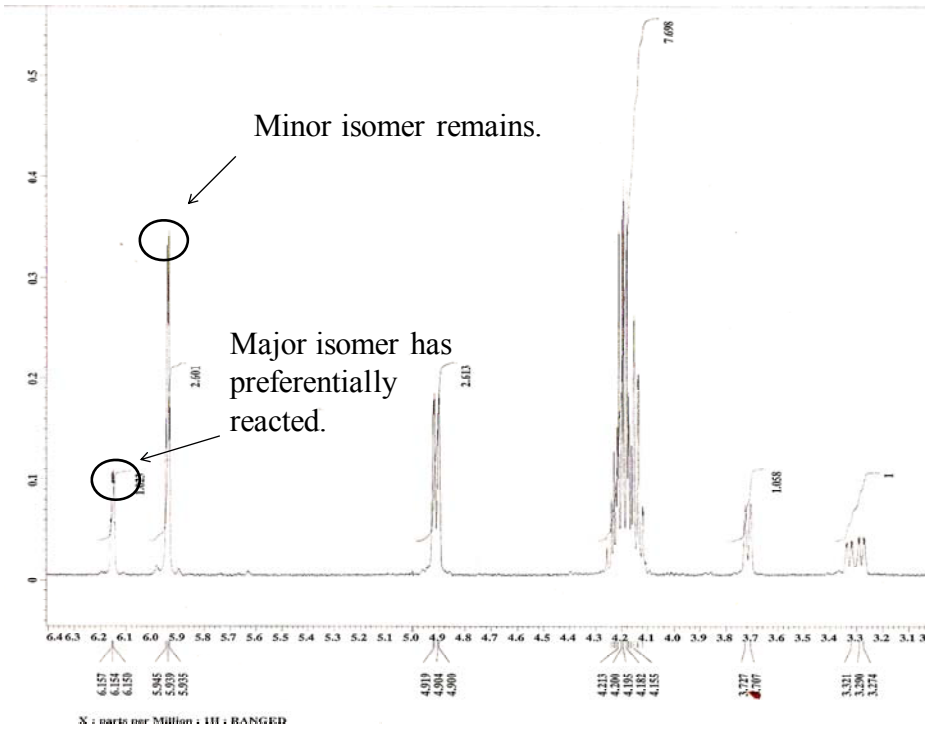

Figure 12: ^{1}H-NMR Spectrum Indicating Major Isomer Reacted

However, following chromatographic purification, it appeared the double bond was reforming while the methyl peak's resonance changed from 1.4 ppm to 2.0 ppm in the NMR spectrum. After full analysis including ^{13}C-NMR spectroscopy, it was concluded that the carbonyl of the lactone ring was being attacked by the cuprate, generating a new methyl group bonded where the lactone carbonyl was to form the lactol shown in **Figure 13**. The work-up resulted in a shift in the carbon-carbon double bond of the conjugated ester. This double bond shift makes it a *tetra*-substituted alkene, which with four other carbon groups bonded is the most stable form possible in the system. After chromatography, the acidic conditions of the column caused the ring to open with the final product containing a methyl ketone, which explains the downfield shift in the methyl carbon bonded to the ketone carbonyl as electron density is pulled from it. The proposed sequence is highlighted in **Figure 13**, with the new methyl addition highlighted in red in **compound 6.**

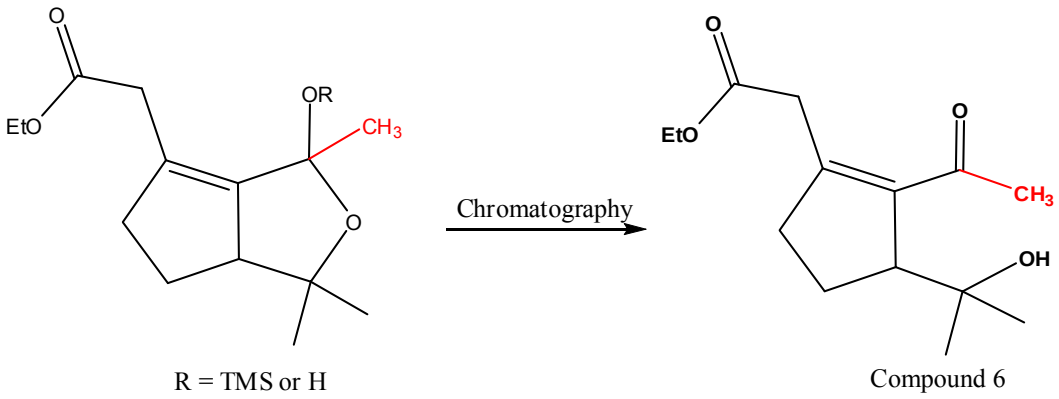

Figure 13: Proposed Cuprate Addition Sequence

The double bond shift is confirmed by the disappearance of the double bond proton from the ^{1}H-NMR spectrum shown in **Figure 14**, which is structurally logical given there are no hydrogens bonded to a *tetra*-substituted alkene. It was reasoned the double bond was still in the molecule because of the presence of two alkene C=C peaks in the ^{13}C-NMR spectrum shown in **Figure 15**. It is known from literature precedent that alkene peaks arise from approximately 100-170 ppm.[10] In this case, the peaks appear at 142.2 and 145.4 ppm, consistent with expected position. The proposed mechanism for the actual reaction is highlighted in **Appendix C**.

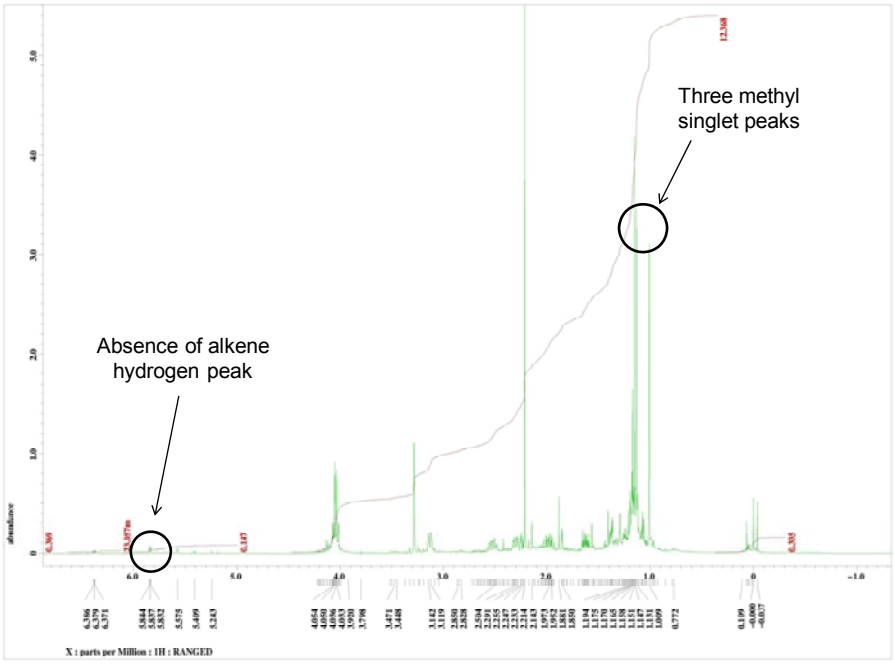

Figure 14: Disappearance of Double Bond Proton with New Methyl Peak

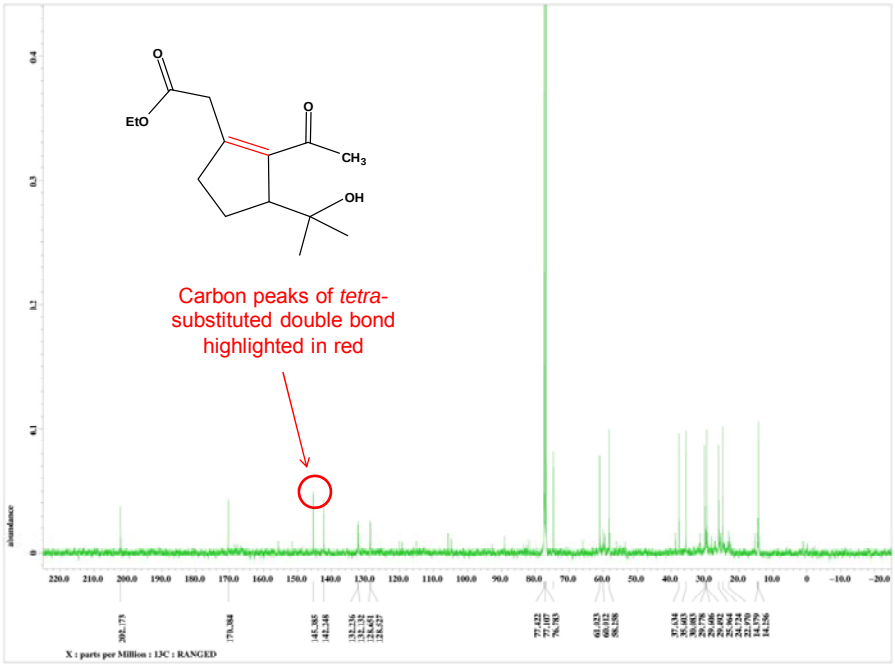

Figure 15: ^{13}C-NMR Spectrum Highlighting *Tetra*-substituted Carbon Double Bond Peaks

This mode of addition of a cuprate to a lactone carbonyl is unprecedented in the chemical literature. It is possible that the TMSCl used to increase the reactivity of the carbonyl is leading to this unanticipated result, as shown in **Figure 38** in **Appendix C.** This transformation would result in three conjugated double bonds. Then, the excess cuprate could attack the most accessible carbon, leading to formation of the lactol after acidic work-up. Depending on the position protonated, this possibility would also generate a *tetra*-substituted carbon-carbon double bond and three methyl peaks in the NMR spectra.

Though previously undocumented in that cuprates generally preferentially attack ketones vice esters, the results present interesting implications both for functionality in bicyclic systems and lactones in general. Since investigation of this mechanism would require extensive time, additional reactions were pursued to introduce alternative stereocenters relevant to dolabellanes. The results and discussion of these reactions follows.

Goal III A. – Catalytic Hydrogenation

The introduction of the alkene bond through the α,β-unsaturated ester produced in step 1 also opens an alternative area of stereochemical exploration. If the double bond could be converted to a single bond with the addition of hydrogen stereospecifically, then methods for creating tertiary stereocenters relevant to dolabellanes could be pioneered. The most common means for adding hydrogen to carbon-carbon double bonds is catalytic hydrogenation.[11] The reaction involves surface chemistry and consists of using any of several precious metals as a template for adsorbing the unsaturated organic compound.[12] Hydrogen gas is also adsorbed onto the surface of the metal. The hydrogens from the metal are transferred to the double bond, creating a single bond. Given the nature of the linear arrangement of the alkene association with

the metal, both hydrogens are usually added to the same side of the organic reagent thus producing a stereospecific alkane.[13]

In this investigation, the pertinent questions were (1) could the alkene be reduced, and (2) would the hydrogen added to the number 4 position be added *cis* or *trans* to the bridgehead carbon. The metals studied were palladium, platinum, and the Raney nickel catalyst. The catalysts themselves are usually absorbed on an inert material, such as carbon, that serves as a storage and delivery mechanism. Since the catalysts are insoluble and remain as solids suspended in the reaction mixture, they act as heterogeneous catalysts. However, certain complexes of transition metal elements are soluble. Known as homogeneous catalysts, they offer an alternative means of producing the tertiary stereocenter. One such catalyst, known as Wilkinson's catalyst, was investigated under several conditions. The solvents used were either ethyl acetate or ethanol. Once the flask had been purged with argon and hydrogen, the hydrogen gas itself was delivered at atmospheric pressure (1 atm) or approximately 60 pounds per square inch (psi) using a pressurized chamber.

Stereochemical specificity was achieved for both heterogeneous and homogeneous catalysis. The general reaction sequence and stereospecific product are shown in **Figure 16**. In the following results presentation, the ^{1}H-NMR spectra are included to demonstrate successful synthesis with the more thorough carbon and two-dimensional results and explanation presented in the NMR section found in **Appendix D**. Nuclear Overhauser effect NMR experiments were used to establish structural stereochemistry at the tertiary stereocenter and are also discussed in the NMR section.

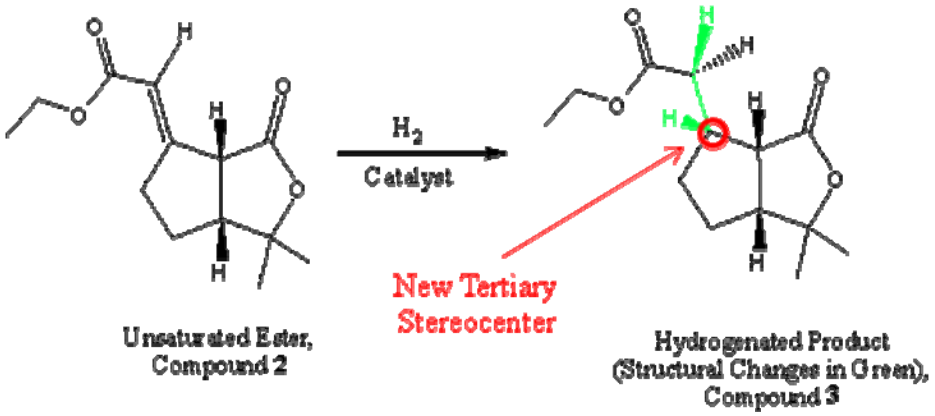

Figure 16: Catalytic Hydrogenation Reaction

The Raney nickel catalyst yielded extremely pure product in a single isomeric form when the reaction was run with ethanol as the solvent, as evidenced by **Figure 17**, which displays the ^{1}H-NMR spectrum of the pure product. An alloy of nickel and aluminum, Raney nickel is produced by using sodium hydroxide to remove selectively most of the aluminum while leaving enough to maintain a porous structure of the catalyst that gives it the large surface area necessary for catalysis.[14] The resulting material is a three-dimensional matrix with nickel as the active constituent present at the surface of the catalytic sites. In this case, the pore size of the catalyst was about 50 μm with a corresponding surface area of 80-100 m^2/g. The catalyst was stored as 50% slurry in water with a basic pH. The yield for the reaction was only 49.6%, but perhaps this number was low because this was the first reaction of its type conducted. Several iterations are usually necessary to bolster yield and optimize the purification steps, such as the Celite filtration used in this procedure.

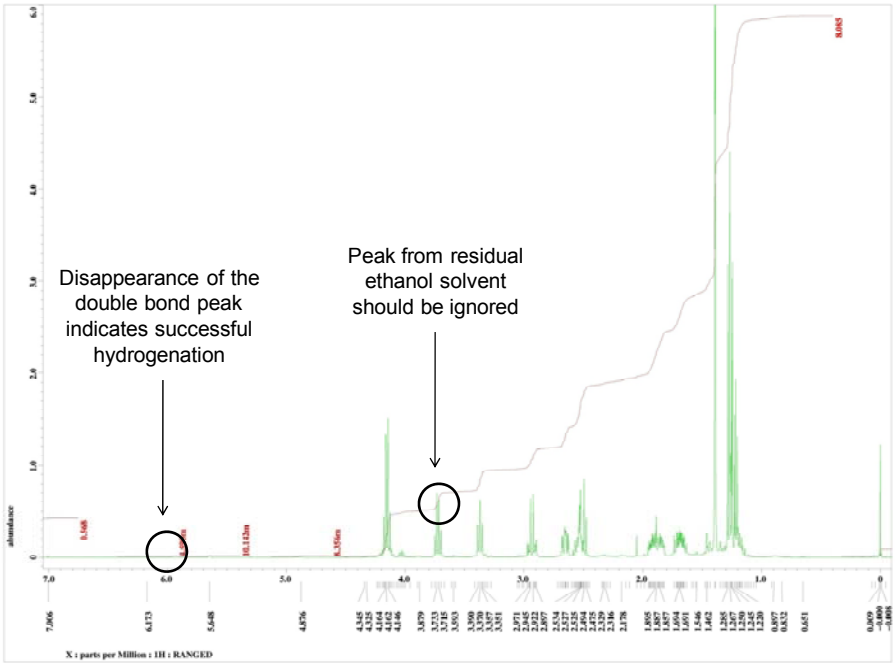

Figure 17: ^{1}H-NMR Spectrum of Raney Nickel Hydrogenation Product

Platinum also gave a single isomerically pure product. As can be seen in the ^{1}H-NMR spectrum shown in **Figure 18**, the spectrum is completely identical to that displayed above with the exception that the ethanol peak has been removed. This provides further confirmation that a single isomer was being synthesized as well as successful the ability of multiple catalysts to uptake hydrogen gas and completely transfer it to the unsaturated double bond. The solvent used in this case was ethyl acetate, which both satisfactorily dissolved the unsaturated carbonyl and suspended the metal catalyst. In fact, ethyl acetate proved to be an excellent solvent for all of the successful catalytic hydrogenation reactions. The yield for the platinum reaction with ethyl acetate was 68.7%, a noted increase over that obtained with the Raney nickel catalyst.

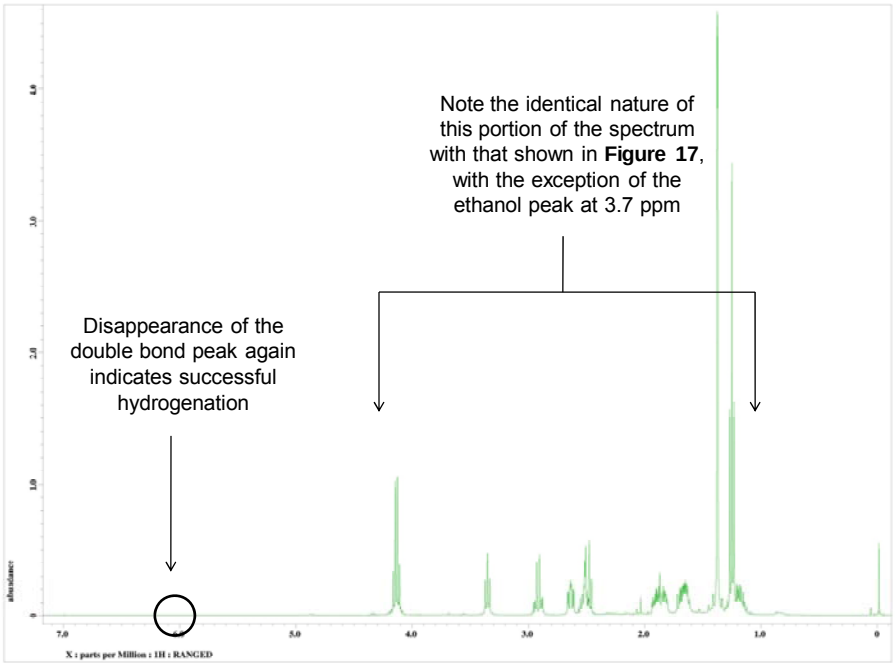

Figure 18: ^{1}H-NMR Spectrum of Platinum Hydrogenation Product

Palladium, however, failed to catalyze a reaction when dissolved in ethyl acetate. As evidenced by the ^{1}H-NMR spectra highlighted in **Figure 19,** both 5% and 10% palladium on carbon clearly did not remove the peak indicative of a carbon-carbon double bond. This was interpreted to mean that the reaction did not proceed at all, given the spectrum matches that of the pure unsaturated ester, **compound 2**. As previously articulated, the absence of this peak at approximately 6.1 ppm was the principal means of characterizing successful formation of the alkane product. It was surmised that increasing pressure and temperature might allow for the better absorption of hydrogen onto palladium catalyst followed subsequently by successful reaction. Thus, 10% palladium on carbon was combined with the α,β-unsaturated ester in 5-ml of ethyl acetate and sealed in a high pressure reaction apparatus. With this configuration, it was possible to raise the pressure to 60 psi of H_2 and the temperature higher than the atmospheric pressure boiling point of ethyl acetate (77°C). Nonetheless, after three days of reaction time, the

alkene stilled failed to be hydrogenated to the corresponding alkane, as evidenced again by ^{1}H-NMR identical to that shown in **Figure 19**.

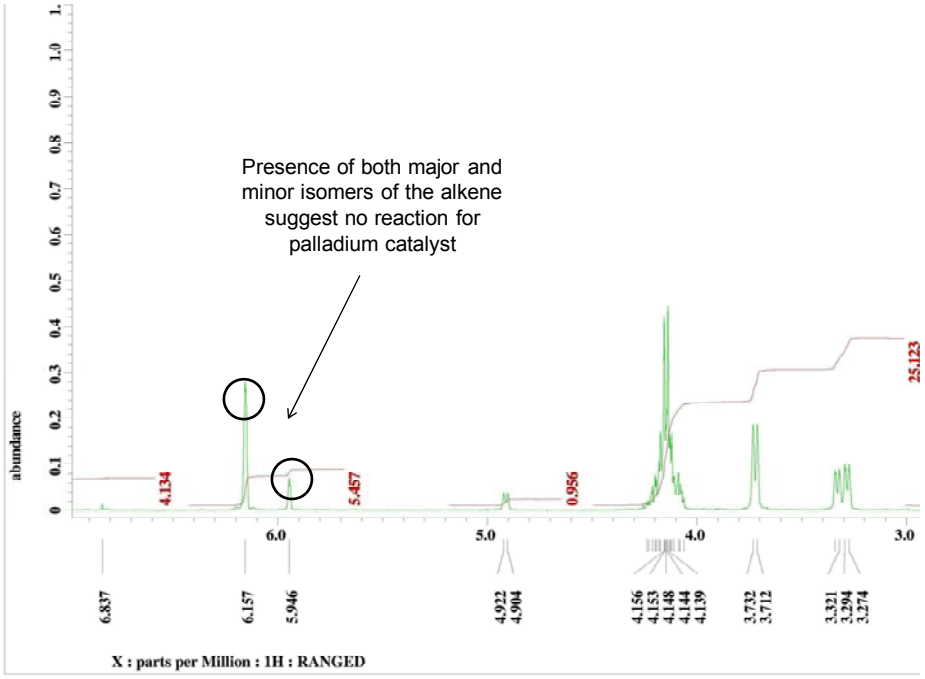

Figure 19: ^{1}H-NMR Spectrum Showing No Reaction for the Palladium Catalysts

Homogeneous catalysis was next investigated. Homogeneous hydrogenations involve a degree of synthetic preparation in order to bestow solubility on the transition metals necessary to affect catalysis. Wilkinson's catalyst, first prepared by Geoffrey Wilkinson in 1966, consists of three phosphine ligands surrounding a central rhodium atom that represents the catalyzing transition metal. In this case, the catalyst had to actually be synthesized following the literature procedure. The phosphine groups enable the compound to be dissolved in organic solvents as well as modify the electronic properties of the metal so that it can coordinate with the unsaturated hydrocarbon bond.[15] After this initial π-complexation of the metal and alkene bond, hydrides are transferred to the double bond followed by reductive elimination, resulting in

complete hydrogenation and dissociation of the new alkane from the metal complex. The structure of Wilkinson's catalyst is shown in **Figure 20**.

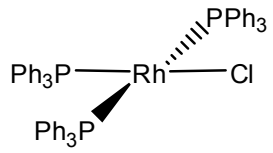

Figure 20: Wilkinson's Catalyst

The initial reaction using ethyl acetate as the solvent was run for three days at room temperature and atmospheric pressure. After filtration, the resulting ^{1}H-NMR spectrum was identical to that presented in **Figure 19**, clearly highlighting the presence of the peak at approximately 6.1 ppm characteristic of the alkene and thus also corresponding to a failed reaction. So, initially, it seemed the Wilkinson's catalyst and thus the attempt at homogeneous catalysis would result in no hydrogenation. However, when run at 60 psi of H_2 and a temperature greater than 77°C for six days, the reaction went to completion with successful hydrogenation and synthesis of the tertiary stereocenter. The ^{1}H-NMR spectrum exactly matched those collected for both the Raney nickel and platinum catalysts. The yield for the reaction was one of the highest in the series as well – 81%. When ethanol was used as the solvent, similarly successful results were observed. The collective and summarized results are presented in **Table 1.**

Table 1: Catalytic Hydrogenation Results

Catalyst	Solvent	Type of Catalysis	Temperature (°C)	Pressure (psi)	% Yield
Raney nickel	Ethanol	Heterogeneous	25	Atmospheric	49.6
5% Palladium on carbon	Ethyl acetate	Heterogeneous	25	Atmospheric	No reaction
10% Palladium on carbon	Ethyl acetate	Heterogeneous	25	Atmospheric	No reaction
	Ethyl acetate	Heterogeneous	>77	60	No reaction
10% Platinum on carbon	Ethyl acetate	Heterogeneous	25	Atmospheric	68.7
Wilkinson's Catalyst	Ethyl acetate	Homogeneous	25	Atmospheric	No reaction
	Ethyl acetate	Homogeneous	>77	60	81
	Ethanol	Homogeneous	>77	60	95

Goal III B. – Grignard Addition:

Grignard reagents are organometallic compounds that are both basic and nucleophilic. It was surmised that the conformational rigidity of the bicyclic starting compound would afford stereochemical addition to the ketone carbonyl. If added in a 1:1 molar ratio, the Grignard might alkylate at the ketone exclusively over the ester carbonyl because of the enhanced reactivity of the ketone group. The proposed reaction is shown in **figure 21.** This would in effect result in the synthesis of a tertiary stereocenter in the form of a stereochemically specific alcohol where the ketone carbonyl was formerly.

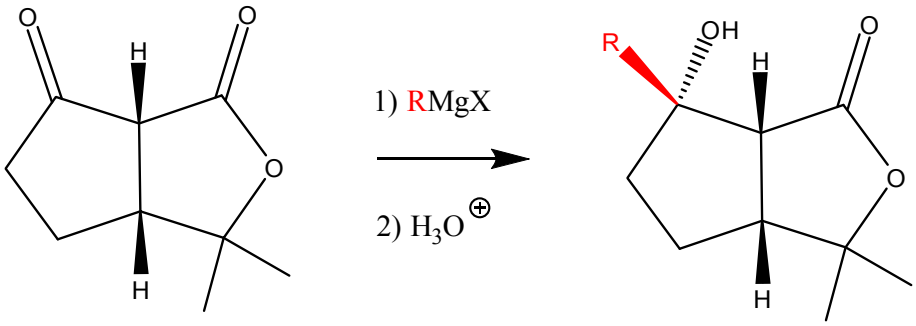

Figure 21: Proposed Grignard Addition

However, it is also known that Grignard reagents are heavily influenced by sterics. Recent examination of the sterics of the bicyclic molecule indicates the bridgehead hydrogen of

the convex face extends above the ketone, meaning Grignard reagents would encounter this hydrogen first and might deprotonate it. In addition, it is known that carbonyl groups increase the acidity of C-H bonds on adjacent carbons relative to carbons on alkyl chains.[16] The presence of a conjugated carbon-carbon double bond and ester on one side and the lactone carbonyl on the other increases the acidity of the bridgehead hydrogen in the middle, as electron density is pulled from the bridgehead carbon weakening the C-H bond and predisposing it to dissociation. As seen in **Figure 22**, the ^{1}H-NMR spectrum collected following reaction at -15°C is identical to that of the bicyclic starting compound, indicating the bicyclic compound was regenerated following the acidic quenching of the reaction and subsequent reprotonation of the bridgehead position. The same results were obtained for Grignard reactions at 0°C and room temperature.

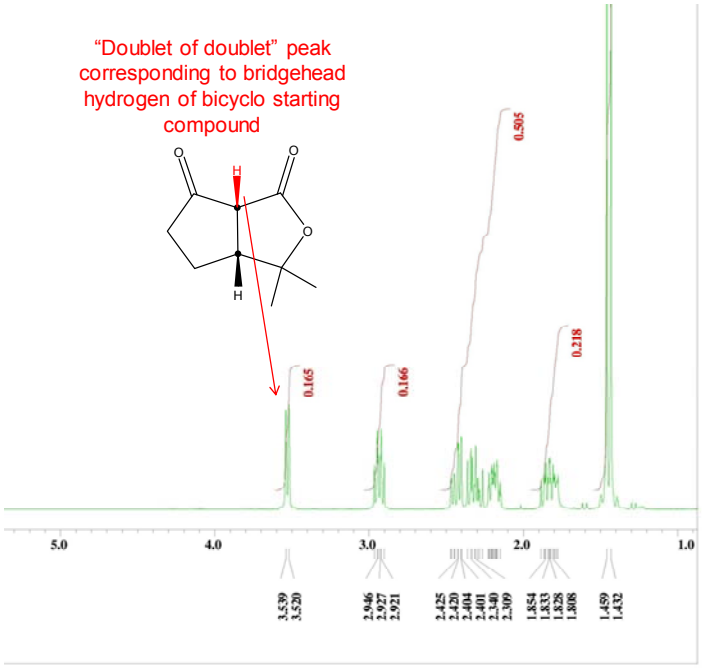

Figure 22: ^{1}H-NMR Spectrum Highlighting Grignard Acting as a Base

It was concluded that by working the reaction up with D_2O the deprotonation of the bridgehead hydrogen by Grignard reagents could be confirmed. D_2O, or heavy water, has deuterium in place of the hydrogens of water. Deuterium has a mass number of 2 because it has one proton and one neutron. By contrast, regular hydrogen has a mass number of 1 because it has one proton and no neutrons. When D_2O is used to reprotonate, the bridgehead hydrogen peak should disappear from the ^{1}H-NMR spectrum because this technique only detects nuclei consisting of the single proton isotope of hydrogen. This is precisely what occurred, as evidenced by the ^{1}H-NMR spectrum shown in **Figure 23**. The crude mixture was analyzed directly by NMR without extraction or solvent evaporation, explaining the THF peak remaining in the proton spectrum.

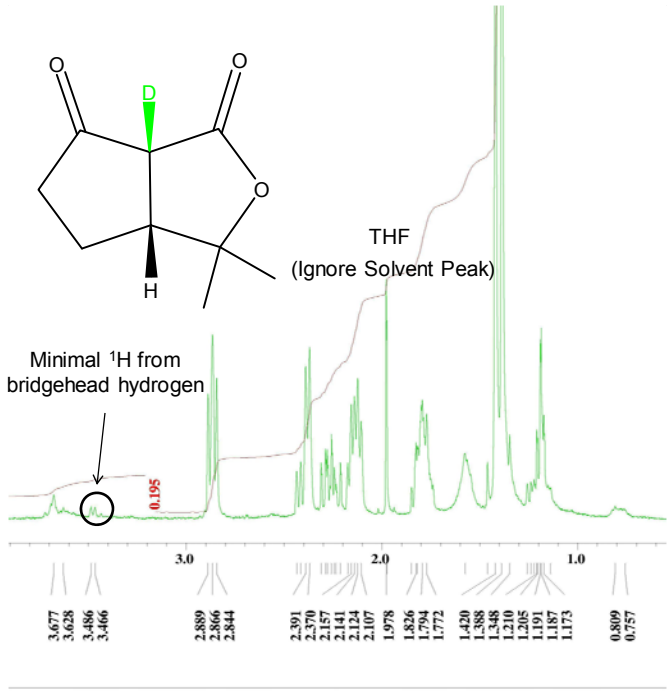

Figure 23: Dueterated Bicyclic ^{1}H-NMR Spectrum

Goal IV – Reduction Reactions

Reduction is a type of reaction whereby electrons are added to an atom or compound. For organic chemists, this often means that the oxygen content of a molecule is decreased while the hydrogen content increases. With respect to the bicyclic starting compound, when the ketone carbonyl is converted to a hydroxyl group, both of these criteria are met. The formerly double-bonded carbon and oxygen become a single bonded alcohol group and a new hydrogen is added to the ring carbon that was a carbonyl group. Now, the carbon of the ring has the potential to become a stereocenter, which has been the focus of this research project, because four different substituents would be attached to the carbon. Specifically, when the ketone carbonyl is reduced, a secondary stereocenter can result. Two isomeric confirmations are possible, as shown in the synthetic scheme presented in **Figure 24.** Methodology was designed that would allow both for the stereochemical implications of the bicyclic framework to be elucidated and to create reaction conditions that would allow for the exclusive synthesis of the stereochemically specific **compound 4**. Fixing the stereochemistry of the carbon with the star in **Figure 24** can thus be extended to relevant dolabellanes by opening up the other ring and modifying the carbon framework. Various borohydride reagents were used throughout the investigation, as they have substantial literature precedence as reduction agents. These reagents are also selective for ketones and will not usually reduce lactones. One stronger reducing agent, lithium aluminum hydride, was used in preliminary reactions and as expected, reduced both carbonyls.

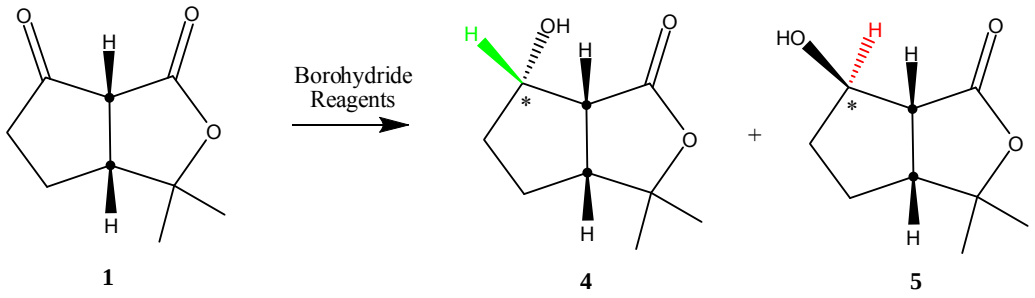

Figure 24: Two Possible Bicyclic Reduction Isomers

To evaluate fully the bicyclic system with respect to reduction reactions, several approaches were pursued that adjusted either reactivity or steric bulk. To begin, three unhindered reagents were used, given their ready availability and comparatively simple handling. Unhindered simply means they do not have any large bulky alkyl or aryl groups extending from them that would preclude attack from the less approachable face of the bicyclic molecule. Due to their comparatively small size and increased reactivity, these reagents were thus not expected to discriminate stereochemically. The first, sodium borohydride, is a weaker reducing agent that generally reduces ketones and aldehydes to alcohols but does not affect reduction of amides, esters, and carboxylic acids. This potential selective ketone reducing power would be of great interest to this project, since the bicyclic motif bears an ester lactone in the right-hand cyclopentane ring. Theoretically, these tendencies would mean that the ring structure would be maintained while the ketone of the cyclopentane ring would be reduced to a tertiary alcohol. It was investigated to see if the reaction would be stereoselective or would fail to select between the two possible isomers. Two different temperatures were explored to determine if lowering the temperature would minimize structural movement, maximize rigidity, and thus increase the likelihood the reaction would be biased towards one stereochemical isomer. The first temperature was room temperature (~25°C), which is often employed in literature references and

usually is sufficient to accomplish the desired reduction. The second temperature, 0°C, was the lowest possible because the reaction was run in an aqueous medium and the freezing point of water necessarily determines the minimum temperature of the reaction mixture.

The isomeric ratio of the reaction was determined by evaluating the integration of the respective ^{1}H-NMR peaks for each stereochemical outcome. As predicted, the unhindered nature of sodium borohydride meant little stereochemical discrimination resulted. In fact, the cousin peaks at 4.55 ppm and 4.45 ppm integrated to values that indicated the former peak constituted 43.84% of the product mixture while the latter represented 56.16% for the reaction run at room temperature. From hindered reduction two-dimensional NMR analysis discussed later, it was determined these peaks represented the new hydrogen added to the carbon of the former ketone carbonyl during the reduction. The peak which integrated to a greater value indeed was that of the more favorable structure with the predicted stereochemistry shown in **compound 4**. Similarly, another set of peaks at 2.75 ppm and 2.58 ppm generated values of 42.41% and 57.59%, respectively. Again, the majority peak corresponded to **compound 4**. This room temperature ^{1}H-NMR spectrum with isomeric ratios shown is presented in **Figure 25.**

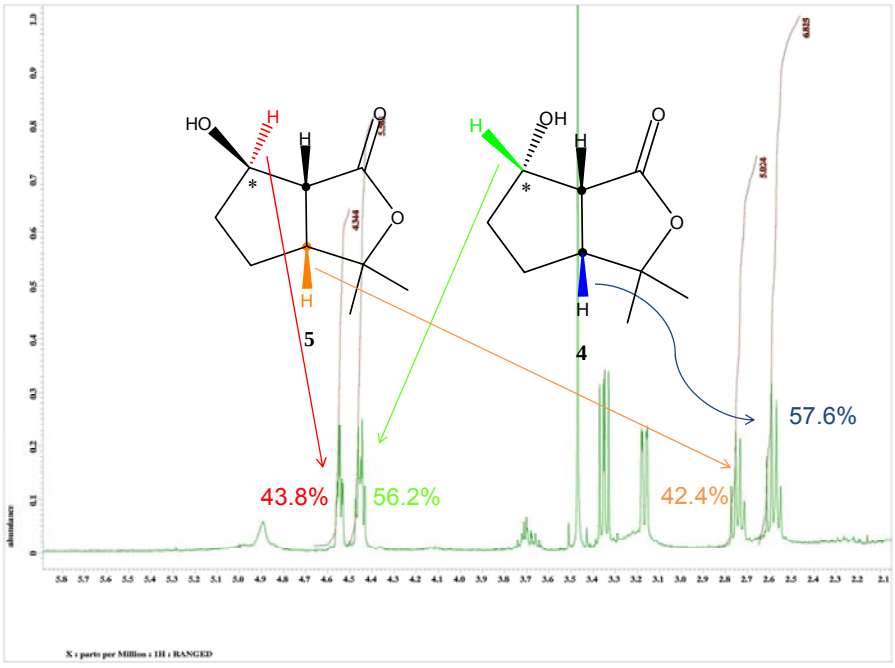

Figure 25: ^{1}H-NMR Spectrum Showing $NaBH_4$ Reduction Ratio @ 25°C

The reaction conducted at 0°C yielded parallel results. Once integrated, the 0°C ^{1}H-NMR spectrum with isomeric ratios calculated generated extremely close numbers to those of the room temperature reaction. In fact, the spectra appeared to be almost identical. Thus, the ratios of each isomer were not dependent on the temperature of reaction. The peaks at roughly 4.55ppm and 4.45ppm gave the following percentages: 43.27% and 56.73%. The peaks at 2.75ppm and 2.58ppm again produced analogous results: 42.76% and 57.24%. As in the reaction run at room temperature, the reduction favored the isomer appearing at the downfield chemical shifts corresponding to **compound 4**. These results corroborated the hypothesis that the isomer of **compound 4** corresponds to the hydride addition that is carried out from the most favorable steric approach.

Sodium cyanoborohydride was the next unhindered reagent explored. This reagent compensates for its diminished reactivity with its small size. Because it is less reactive, it will in

theory preferentially attack from the most energetically favorable position. In other words, though the reaction will proceed slower than conventional sodium borohydride at room temperature, it should nonetheless demonstrate more selectivity. According to literature precedents, the rate of the reaction can be greatly enhanced by decreasing the pH. Following this recommendation through the judicious use of methanolic-HCl, the reaction time was narrowed to approximately one hour.[17] As predicted, the reaction was indeed more selective than the sodium borohydride counterpart. For example, the isomeric peaks at 4.45 ppm (**compound 4)** and 4.55 ppm (**compound 5**) integrated to 66.0% and 34.0%, respectively. Similarly, the isomeric peaks at 2.58 ppm (**compound 4)** and 2.75 ppm (**compound 5**) integrated to respective values of 61.4% and 38.6%. These integrated ratios are shown in the ^{1}H-NMR spectrum of **Figure 26**. So, indeed, the isomer of **compound 4** was shown through further substantiation to be the product of the more favorable approach due to the sterics and rigidity of the bicyclic compound.

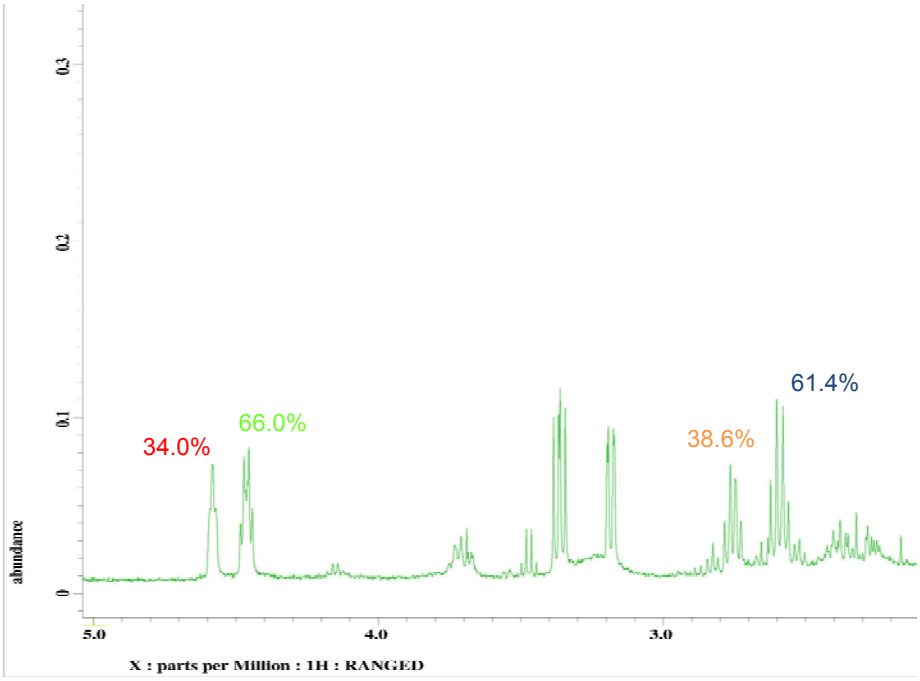

Figure 26: ^{1}H-NMR Spectrum Ratios for $NaCNBH_3$ Reduction

The last unhindered reagent explored was lithium aluminum hydride, or LAH. This reducing agent is more powerful than the sodium borohydride counterpart, by virtue of the weaker aluminum-hydride bond in comparison to the boron-hydride bond. This relative bond weakness more readily predisposes LAH to lose its hydride constituents to electrophilic centers such as those in carbonyls. In fact, lithium aluminum hydride will reduce both esters and ketones. The structure of the compound is shown in **Figure 27**.

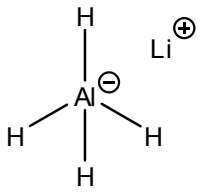

Figure 27: Structure of Lithium Aluminum Hydride

Because of its enhanced reactivity, it was expected that LAH would not only reduce the ketone to a secondary alcohol but would also reduce the lactone to two primary alcohols. This dual reduction is precisely what was observed. The reaction leading to the completely reduced compound is shown in **Figure 28** with changes indicated in red.

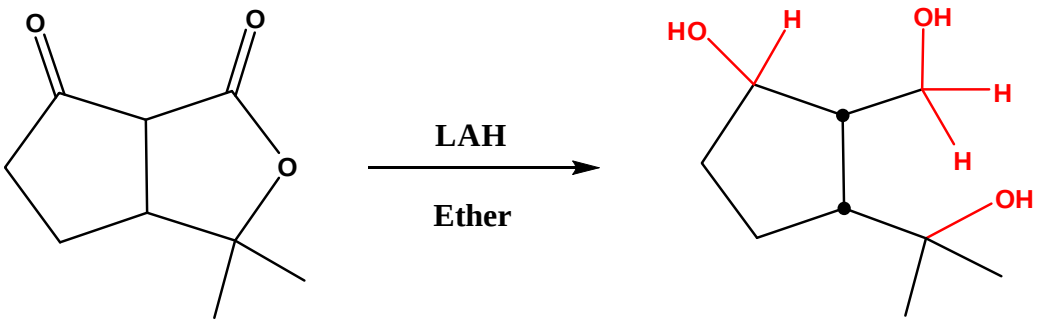

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Figure 28: Complete Reduction of Bicyclic Compound with LAH

To enhance the selectivity of reduction, the sterically hindered reagents were predicted to have much more selectivity in the reduction product. The first hindered reagent, known as K-selectride, has a chemical name of potassium tri-*sec*-butylborohydride. The bulky size of the reagent naturally discriminates it towards the production of a single isomer because it can only approach the bicyclic compound from one side.[18] Its structure is shown below in **Figure 29.**

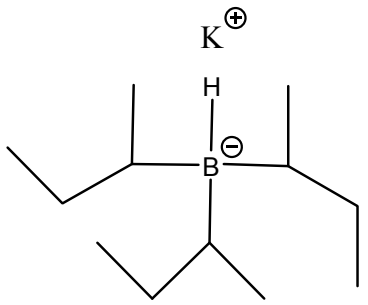


Figure 29: Structure of K-selectride

The first procedure consisted of lowering the temperature to -78°C and conducting the reaction in an aprotic solvent. However, the oxidative work-up designed to remove the borane byproducts was too vigorous, as the hydrogen peroxide employed converted the organic product to carbon dioxide evidenced by rapid gas evolution.[19] Switching to a more gentle work-up with ammonium chloride to eliminate any remaining anions followed by chromatography to separate the alkylborane byproducts resulted in the isolation of a single purified reduction isomer, as highlighted in the ^{1}H-NMR spectrum shown in **Figure 30**.[20] Note that only a single peak appears at both 2.597 ppm and 4.454 ppm, indicating the product was 100% **compound 4**.

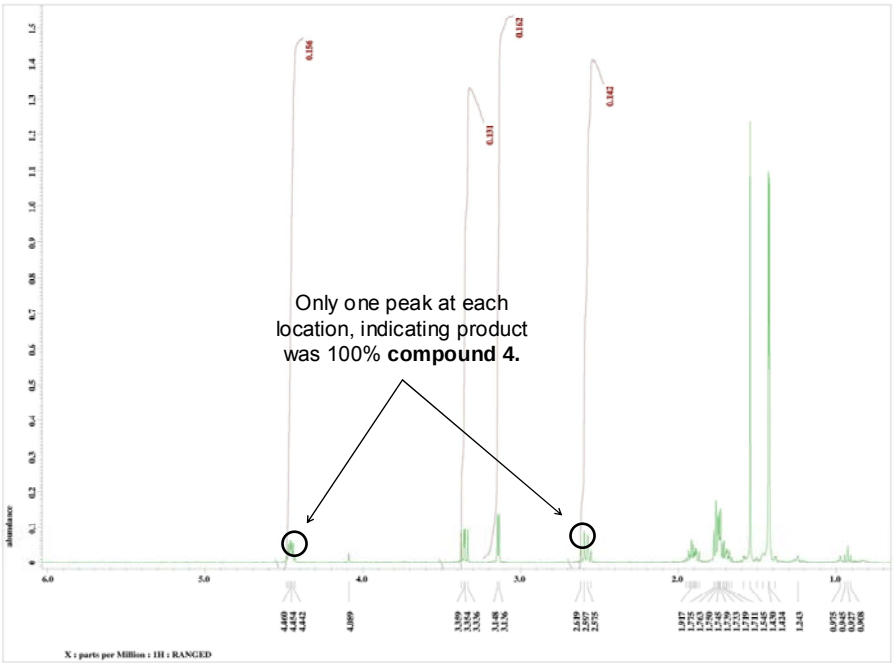


Figure 30: ^{1}H-NMR Spectrum of Hindered Reduction Product

The K-selectride reaction served to verify that a secondary stereocenter pertinent to dolabellanes could indeed be synthesized, purified, collected, and analyzed by NMR. However, the yield was fairly low because of imperfect chromatography conditions, as explained in the experimental appendix. To both improve yield and to expand the hindered reagent scope of the project, L-selectride in the same concentration with the same reaction procedure was studied. L-selectride is the name for lithium tri-*sec*-butylborohydride which has a very similar structure to K-selectride. Its structure is exhibited in **Figure 31.** The chromatography conditions were altered such that a 98.8% yield of **compound 4** was obtained. **Compound 4** was determined to be the product of the L-selectride reaction because the ^{1}H-NMR spectrum for the L-selectride product was essentially identical to that shown in **Figure 28** for K-selectride These results highlight the utility of the hindered reduction reagents both for stereospecificity and nearly quantitative yield. The overall results are summarized in **Table 2** on the following page.

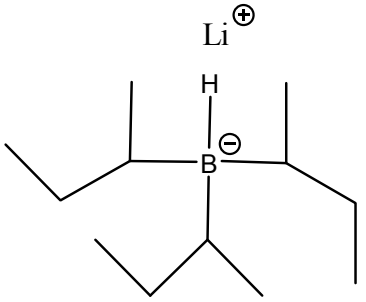


Figure 31: Structure of L-selectride

Table 2: Borohydride Reduction Results

Reducing Agent	Structure Type	Temperature (°C)	Isomeric Ratio (4:5)	Time (hours)
Sodium Borohydride	Unhindered	25	57/43	0.5
Sodium Borohydride	Unhindered	0	57/43	0.5
Sodium Cyanoborohydride	Unhindered	25	66/34	1
L-selectride	Hindered	-78	100/0	2.5
K-Selectride	Hindered	-78	100/0	2.5

Stereochemical Determination:

For **compound 3** and **compound 4**, the stereochemistry was assigned using a technique known as one-dimensional nuclear Overhauser effect (nOe) spectroscopy. This is a difference technique that shows enhancement of peaks when they are *cis* to one another. Some of the technique specifics are presented in the NMR section in **Appendix D**. Because the ^{1}H-NMR peaks for the new hydrogens added in both catalytic hydrogenation and hydride reduction were indeed enhanced when the bridgehead hydrogen was tested, it was conclusively determined that addition occurred *cis* to the bridgehead hydrogens of the bicyclic compound.

CONCLUSIONS AND FUTURE WORK:

When the project was first designed, four synthetic methodology goals were articulated with emphasis on different degrees of stereocenters:

I. The conversion of the ketone of a rigid bicyclic system to an alkene, using either a Horner-Emmons reaction or a Wittig reaction.

II. The synthesis of a quaternary stereocenter using cuprate reagents to accomplish 1,4-conjugate addition of the alkene synthesized in goal I.

III. The synthesis of tertiary stereocenters using the following two methods:

   A. Catalytic hydrogenation of the aforementioned alkene

   B. Organometallic addition to the ketone of the bicyclic system.

IV. The synthesis of a secondary stereocenter at the ketone of the bicyclic system through the use of reduction reagents.

In the following section, the conclusions from each one of these objectives will be articulated with the results summarized and future work specific to each goal presented.

Goal I:

The first synthetic step was completely carried out and the product characterized successfully. Although the Horner-Wadsworth-Emmons mechanism did produce the desired α,β-unsaturated ester, the Wittig reaction provided better overall results. Once chromatography conditions were achieved, the first synthetic product, **compound 2**, was obtained readily in pure form with predictable yield. The unsaturated ester is being submitted for elemental analysis to confirm its molecular composition. Future work with respect to the first synthetic step includes the possibility of varying the alkyl portion of the ester introduced along with the double bond. Also, syntheses of ketones and aldehydes rather than esters should be explored.

Goal II:

From pilot reactions with phenyl cinnamate, it was demonstrated that 1,4-conjugate addition to an α,β-unsaturated ester using cuprate reagents could be accomplished. The successful product was characterized using GC/MS. The homocuprate used in all investigations was lithium dimethylcuprate. Formation of the cuprate was evidenced by a characteristic color change that occurred when the second methyl group of the complex was added. Although it was predicted that the cuprate would add to the number 4 position of the α,β-unsaturated ester introduced into the bicyclic system as cited in literature precedent and observed in pilot reactions, the sterics and strain of the bicyclic network generated an entirely novel reaction pathway. By way an unprecedented mechanism, the cuprate actually attacked the lactone ring rather than the ketone of the bicyclic template. In the course of the work-up, the alkene bond rearranged to form a stable *tetra*-substituted double bond. Ultimately, the lactone was opened up with the formation of a methyl ketone group. The conclusions were determined from ^{1}H and ^{13}C-NMR spectroscopy.

Future work with respect to the quaternary stereocenter involves continuing to pursue 1,4-conjugate addition with different conjugated systems, such as α,β-unsaturated ketones and aldehydes. The nature of the nucleophile could be changed to adjust reactivity. For example, organozincs, copper-catalyzed Grignard reagents, and heterocuprates could be explored. Moreover, the full implications of the unique lactone ring-opening should be studied to gain a thorough understanding of how the bicyclic system directs to that particular electrophilic center.

Goal III - Part A:

The creation of a tertiary stereocenter was also accomplished with great success in the second semester of the research project. Catalytic hydrogenation of the α,β-unsaturated ester synthesized in Goal I was the reaction pathway that afforded the stereospecificity. **Compound 3**, showing the all *cis*-hydrogen configuration, was the only isomer produced with 100% exclusivity. Success was achieved with multiple metals in both heterogeneous and homogeneous catalysis. Raney nickel and platinum both demonstrated utility as far as heterogeneous catalysis is concerned. Wilkinson's catalyst also proved to be an excellent source of homogeneous catalysis at elevated temperature and pressure. Both ethyl acetate and ethanol proved to be effective solvents for carrying out the reactions. The primary analytical means of evaluating the reaction was the disappearance of the alkene double bond hydrogen peak located at 6.1 ppm on the ^{1}H-NMR spectrum.

The results are summarized in **Table 1** presented in the discussion section. Although palladium did not achieve hydrogenation, this was most likely due less to its inability to catalyze the reaction as to the possibility that the catalyst was spoiled and no longer viable chemically. The remainder of the results clearly demonstrated the ability of the bicyclic framework to direct hydrogen addition stereospecifically. This is important for a number of dolabellane compounds with similar tertiary stereocenters. The scopes and limitations of the catalysis could be explored further by widening the range of solvents used, as well as investigating additional precious metal catalysts and homogeneous catalysts.

Goal III – Part B:

When Grignard reagents with small steric hindrance, such as methyl Grignard, were added to the bicyclic starting compound, interesting results were obtained. The hydrogen between the two carbonyls at the top bridgehead position is especially acidic. Due to its accessibility, this hydrogen is particularly susceptible to basic removal. Thus, the Grignard reagents acted as bases, removing the proton from the bridgehead rather than behaving as nucleophiles and adding to the ketone to produce a tertiary alcohol. These results were confirmed analytically by working the reaction up with D_2O rather than regular aqueous solutions and noting the disappearance of the bicyclic bridgehead hydrogen peak from the ^{1}H-NMR spectrum. These results led to the conclusion that examination of other less basic organometallics will be necessary to prepare tertiary alcohols.

Goal IV:

Sodium borohydride reduction reagents exhibited the potential to reduce selectively the ketone carbonyl of the bicyclic compound while not reacting at the lactone ester of the other ring. This reactivity proved to be invaluable to stereospecific reduction once steric bulk was introduced into the reducing reagents. Lithium aluminum hydride, on the other hand, reduced every carbonyl in the molecule to the corresponding alcohol, as expected due to its known increased reactivity and strength as a reducing agent.

To evaluate the stereochemical implications of borohydride compounds, reagents with no steric hindrance were reacted first at several temperatures to determine the isomeric ratio of **compound 4** to **compound 5**. These reagents were termed unhindered reducing agents. The stereoisomer of **compound 4,** shown with *S*-configuration, was favored indicating indeed the

face with both bridgehead hydrogens projecting offers the most energetically feasible approach. Once significant steric hindrance was introduced into borohydride reagents through the use of L-selectride and K-selectride, steric approach control was achieved with 100% precision, as **compound 4** was the sole product of the reduction. These significant results show that the bicyclic template offered a pathway for stereospecific reduction and provided access to synthesis of secondary stereocenters relevant to the dolabellane family of compounds. The results of the investigation are presented in **Table 2** in the discussion section.

APPENDIX A – EXPERIMENTAL:

I. Glassware:

In general, all glassware was dried for at least 24 hours in an oven at approximately 120°C. Before use, glassware was transferred to a desiccator, which maintains a dry atmosphere yet allows instruments or compounds to cool.

II. Solvents:

Anhydrous solvents were obtained from a solvent manifold, which acts as a reservoir for storing drying solvents. Dry refers to solvents that are anhydrous, or devoid of water. The solvents were dispensed into Schlenk flask. Other solvents were reagent grade and used as supplied by the manufacturer without further purification.

III. General Procedure:

The dry starting compound, dried in a separate desiccator used only for reagents, was added to the cooled glassware. From here, the glassware would be clamped into place in the hood and immediately put under an inert gas. Inert gas prevents undesired deterioration of the compounds, many of which are sensitive to both water and air. Argon was the inert gas of choice for several reasons. One, as a noble gas, it is extremely unreactive. Two, argon is heavier than air and thus acts as a gaseous blanket, providing a protective layer above the reaction mixture. Using Luer-lock syringes stored in the glassware desiccator and needles dried in the oven, the solvents to be used were withdrawn from the Schlenk flasks used to maintain dry conditions. Via injection through a rubber septum, the solvents and reagents were then introduced into the system. Magnetic stir bars were used to mix the reaction.

Specific procedural details are described for each synthetic step. The first synthetic reaction consisted of reacting the starting material, bicyclic-[3.3.0]-octane, with an alkene introducing reagent, either the appropriate Wittig or Horner-Wadsworth-Emmons compound. After properly combining the constituents, the round-bottom flask was heated to boiling in a process termed refluxing. An instrument known as a reflux condenser permits water to circulate above, thereby condensing any solvent that enters the gas phase, and keeps the system volume constant. When the reaction is complete, as determined by TLC (see chromatography discussion in **Appendix E**), the solvent was removed by rotary evaporation. In order to separate the desired product from the phosphine oxide byproduct and purify the product, the reaction mixture was dissolved in a minimal volume of dichloromethane and placed on a flash chromatography column (see **Appendix E**). Having collected the appropriate fractions, the solvents from the chromatography are again removed by evaporation, leaving the desired α, β-unsaturated ester.

The second synthetic step consisted of reacting the α,β-unsaturated ester with an alkylating reagent. Usually, the alkylating reagents were especially reactive. Thus, all solvents had to be degassed to remove oxygen, as well as dried. A procedure known as free-pump-thawing was used to remove any dissolved gasses from the solvents. First, the solvent was frozen by pouring liquid nitrogen around the Schlenk flasks, themselves held in a Dewar flask. Then the flasks were evacuated. Lastly, the solvent was thawed with a heat gun releasing dissolved gases. The sequence was repeated three times, followed by the introduction of argon into the flask. Special care had to be used when injecting the alkylating reagent. Methyl lithium, for example, reacts immediately when exposed to air. Hence, it was necessary to introduce argon to the sealed bottle of methyl lithium, withdraw the desired amount of reagent via syringe,

and inject it with extreme care into the flask containing the copper(I) compound to ensure proper transfer.

Another unique experimental parameter of the latter synthetic step was the temperature of the reaction. Cuprate reagents are unstable above 0°C and optimal conditions with various solvents necessitate temperatures as low as -100°C. Obtaining temperatures this low was accomplished by combining various solvents with cooling agents, which lowers the freezing point or brings the cooling bath to the freezing point of the primary solvent itself. For example, the freezing point of water was depressed to -15°C by mixing ice and ethanol. The freezing point of acetone, -78°C, was achieved by mixing acetone with liquid nitrogen.

In some cases, extractions were performed to separate the organic products from the aqueous additions used to remove reaction by-products or modify the product to its final form. Liquid-liquid extractions were conducted for both the Horner-Wadsworth-Emmons reaction and the 1,4-conjugate addition reaction with cuprate reagents. Extractions were also necessary for all the reduction reactions because aqueous work-ups were required to protonate the intermediate alkoxide group synthesized. Ether served the role of the organic phase, where the desired organic products preferentially dissolve in the case of the Horner-Wadsworth-Emmons (HWE) and conjugate addition reactions. In the case of the reduction reactions, dichloromethane served as the organic phase. The organic layers of three such extractions were combined and dried with magnesium sulfate, which has a high affinity for water and pulls out any remaining water from the extraction. The solid white magnesium sulfate was added until clumps no longer form, which is an indication that all water has been removed. Lastly, the solution was filtered to remove the drying agent and the organic solvent was removed by rotary evaporation.

IV. Wittig Reaction:

All glassware was dried at 120°C for 24 hours. The Wittig reagent was first purified by dissolving 15 g of the phosphonium salt in 100-mL of water and 90-mL of toluene in a 250-mL round-bottom flask. Phenolphthalein indicator was added. Sodium hydroxide (10%) was added until the solution turned pink, indicative of basic pH.[21] Litmus paper was used to confirm. The toluene ($\rho = 0.93$) layer was separated off the top and dried over $MgSO_4$ and filtered. As much toluene as possible was removed by rotary evaporation. Ether was added and the purified alkene was recrystallized and dried by vacuum.

To a dried three-neck round-bottom flask was added 2.5 g (5.82 mmol, 2 equivalents) of the purified Wittig from above and 15-mL of anhydrous dichloromethane. A mass of 0.5 g (2.97 mmol) of the bicyclic starting compound was added to the solution, which then turned light orange. The solution was put on reflux under Argon for 24 hours, after which time the solution appeared a deep, dark orange color. Chromatography was performed to remove triphenyl phosphine oxide. The crude mixture was dissolved in a minimal amount of dichloromethane before transferring to column. A representative yield was 57.9%.

V. Horner-Wadsworth-Emmons Reaction:

To 12-mL of anhydrous THF in a dry 50-mL round bottom flask was added 0.688g (5.95 mmol) of potassium tert-butoxide. The flask contained a magnetic stir bar and was stoppered with a rubber septum connected via a needle to a positive pressure of argon. The mixture was stirred for 10 minutes at room temperature until the salt was completely dissolved. The alkoxide solution was added to a stirred solution of triethyl phosphonoacetate (1.34g, 5.95 mmol) in 6-mL of anhydrous THF at 0°C in a separate round bottom flask connected to a positive pressure of

argon, producing a light yellow color. In another flask, 0.250 g (1.5 mmol) of bicyclic was dissolved in 6-mL of anhydrous THF. The solution containing the bicyclic compound was added drop-wise via a syringe to phosphonoacetate anion solution and stirred for 20 minutes at 0°C. After allowing it to warm to room temperature, the orange mixture was poured into 25-mL of ether and 25-mL of saturated ammonium chloride in a separatory funnel. The aqueous layer was extracted three times with 15-mL of diethyl ether. The organic layers were combined and dried over magnesium sulfate. The solvent was removed on a rotary evaporator. Purification was performed by pouring over silica gel and filtering for an yield of 60.1%.

VI. 1,4-Conjugate Addition Reaction:

A. Pilot Reaction 1:

Glassware was dried as before. Copper(I) iodide (0.762g, 4 mmol) was added to 5-mL of degassed anhydrous ether under argon in a 50-mL round-bottom flask. The slurry appeared metallic gray in color. The mixture was stirred and the solution cooled to -15°C using an ethanol/ice mixture in an insulated Dewar flask. The temperature of the bath was monitored with a thermometer. Via an air-tight Luer lock syringe, methyl lithium (1.6M, 5-mL, 8 mmol) was injected into the reaction mixture. Immediately upon addition, a yellow precipitate formed indicative of methyl copper. After approximately fifteen minutes, the solution transitions to a cloudy, nearly colorless solution. This is the visual characteristic of successful production of the cuprate reagent lithium dimethylcuprate.[22] Using a small 1-mL glass syringe, 2-cyclohexenone (0.350-mL, 3.64 mmol) was added in order to affect a 1.1:1 molar ratio of organocuprate to conjugated enone. The reaction was allowed to stir for 18 hours and turned yellow-green. Hydrochloric acid (4-mL, 2M) was added until the pH reached 4, as determined with litmus

paper. This mixture was extracted with ether (3 x 15-mL) in a separatory funnel, followed by drying of the combined organic layers with magnesium sulfate and filtering. The solvent was removed on the Rotary evaporator leaving the product as yellow oil. Typical yields were around 55%.

B. Pilot Reaction 2:

Another pilot reaction was performed with a representative α, β-unsaturated ester in order to more closely model the electronic features of the system of interest. In this case, phenyl cinnamate was the compound of choice; its structure is given in **Figure 32.**

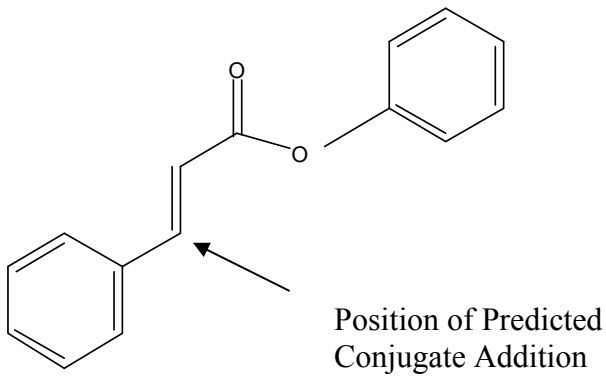


Figure 32: Structure of Phenyl Cinnamate

Procedurally, the experiment resembled that of 2-cyclohexenone with two exceptions. First, a 1.2: 1 molar ratio of organocuprate to α, β-unsaturated ester was employed. Thus, 0.574 g (3.01 mmol) of copper (I) iodide was reacted with 5-mL of methyl lithium (1.6M, 8 mmol). Second, trimethylsilyl chloride (0.51-mL, 0.434 g, 4 mmol) was added at -78°C to the cuprate reagent. Phenyl cinnamate (0.74g, 3.3 mmol) was then added and the mixture was allowed to warm to room temperature slowly. A deep shade of yellow permeated the solution. Ammonium

chloride was added to quench after 18 hours of stirring. The reaction mixture was extracted with diethyl ether (3 x 15-mL) and dried with magnesium sulfate as usual.

C. Addition to **Compound 2**:

The actual reaction with the α, β-unsaturated ester of **compound 2** was conducted on a much smaller scale. Copper(I) iodide (0.288 g, 1.5 mmol) was dissolved in 3-mL of degassed anhydrous ether under argon. Methyl lithium (1.6M, 1.88-mL, 3 mmol) was injected as before and the characteristic color progression observed. TMSCl (0.19-mL, 1.5 mmol) was added at -78°C.[23] The α, β-unsaturated ester (0.3g, 1.26 mmol) was added and the reaction mixture was again allowed to cool slowly to room temperature and stirred for 18 hours. The work-up followed that of the phenyl cinnamate reaction.

An alternative procedure was carried out on a 50 mg scale using a different copper(I) reagent. Copper(I) bromide-dimethyl sulfide (0.0518 g, 0.252 mmol) was dried in a desiccator, purged with argon, and dissolved in 3-mL of anhydrous degassed ether. Temperature and trimethylsilyl chloride conditions were also maintained in this reaction sequence. Time of reaction was reduced to 2 hours and immediately hydrolyzed with hydrochloric acid (2M) until the pH was 4 as determined with litmus paper. Extractions were performed with diethyl ether. Ultimately, the reaction was taken to -15°C immediately after formation of the cuprate and allowed to react for approximately 30 minutes. Then, it was taken to 0°C in an effort to provide the highest temperature where the cuprate would be stable to enhance reactivity. The crude product was chromatographed on silica gel using ethyl acetate/hexane as eluent. Based on ^{1}H and ^{13}C NMR spectroscopy, the major product was identified as **compound 6**, the structure of which is presented on page 25.

VII. Catalytic Hydrogenation:

A. Heterogeneous Catalysis Procedure:

A three-necked flask complete with magnetic stir bar was dried as done previously. To the flask was added 120 mg (0.504 mmol) of **compound 2**. The flask was then flushed with argon to remove atmospheric water and oxygen. The solid ester was then dissolved in 5-mL of solvent. Solvents were varied by catalyst as shown in **Table 1.** The first catalyst employed was 5% palladium on activated carbon. Approximately 10 mg of catalyst were used with the consideration that a slight excess would probably not detrimentally affect the reaction given the nature of the precious metal as a catalyst and not a reagent for consumption. Similar reasoning was used in determining the mass of Raney-Nickel and 5% platinum on activated carbon needed.

Balloons were used to collect and deliver the hydrogen gas in the reaction vessel. The balloons were filled with gas from a pressurized tank containing hydrogen. Via a needle injection apparatus, the gas was introduced into the sealed flask. The mixture was flushed with hydrogen three times to saturate the interior of the flask. Then, the balloon was filled, inserted, and allowed to equilibrate at 1 atmosphere pressure in order to maintain hydrogen volume, as some of the gas invariably escapes. In some cases, the reaction was run at a higher pressure (60 psi) and a higher temperature in order to drive reaction. This was accomplished through the use of a sealed apparatus with a pressure gauge and volume increments labeled.

The reaction was typically run for approximately 72 hours. TLC was employed to verify reaction progress. Following reaction completion, the reaction contents were filtered through Celite, a predominantly silica-based filtration aid with high porosity that effectively removes the precious metal catalyst and activated carbon mixture while allowing the reaction product to pass through with the organic solvent. The Celite was washed with the same organic solvent used for

the experiment so as to ensure maximum recovery of the compound of interest. From there, the organic solvent was removed on the Rotary Evaporator – leaving the hydrogenated bicyclic ester of **compound 3** as a single pure isomer.

B. Homogeneous Catalysis Procedure:[24]

To begin, the tris(triphenylphosphine)chlororhodium(I) compound, $RhCl(PPh_3)_3$, was prepared. A 6 molar excess of triphenylphosphine (15 g, 57.3 mmol) was dissolved in 350-mL of ethanol and heated to reflux. Separately, rhodium trichloride (2 g, 9.56 mmol) was dissolved in 70-mL of ethanol and heated on a hot plate. When each mixture was fully dissolved, the two were combined and heated to reflux for 1 hour. A vacuum filtration apparatus was configured on a hotplate. The solution was filtered hot and washed with anhydrous, degassed ether leaving the burgundy-red crystals characteristic of the Wilkinson's catalyst complex. Using the rhodium trichloride as the limiting reagent, the yield was 68.72%. Because the catalyst is hygroscopic, it was necessary to store it in a desiccator to prevent it from absorbing water. The experiments themselves were conducted in virtually the same manner as the heterogeneous catalysts using the balloon configuration, solvent selection, and Celite filtration. The reaction was also conducted at higher temperature and pressure in the sealed apparatus previously used. In this case, the reaction was allowed to proceed for six days.

VIII. Borohydride Reduction Reactions:

A. Reduction with Sodium Borohydride:

As a separate mixture, a solution of 1.4 g (0.037 mol) of $NaBH_4$ in 2-mL of 2M NaOH diluted with 18-mL of water was prepared. The combined sodium borohydride-base solution was labeled and stored.

The bicyclic system in a mass of 0.11 g (0.655 mmol) was dissolved in about 1-mL of methanol in a 50-mL round bottom flask. The reaction was carried out at either 25°C or 0°C. To this mixture was added 0.5-mL of the $NaBH_4$ solution prepared previously. Though the literature called for only 0.12-mL, it was desirable to use an excess of the reducing agent to ensure the reaction went to completion.[25] The reaction was allowed to proceed for three minutes, at which time 2-mL was placed in a vial and a micro-extraction was performed with ethyl acetate as the organic phase. Using a needle and syringe, 1-μL of the organic phase was injected into the GC/MS to monitor the course of the reaction. The presence of a molecular ion peak at 170 (the product has a molar mass of 170 grams/mole), 155 for the loss of a methyl fragment, and 152 for loss of the new hydroxyl group that weighs 18 g/mol affirmatively demonstrated the reaction had indeed gone to conclusion. The purpose of the reaction was only to assign isomeric ratios so a quantitative percent yield was not obtained. Rather, the reaction mixture was extracted with ethyl acetate, dried with $MgSO_4$, and evaporated to remove the organic solvent. The unpurified product was analyzed using ^{1}H-NMR and the peaks of interest were integrated for calculation.

B. Reduction with Sodium Cyanoborohydride:

Acetyl chloride in a volume of 800-µL was combined with 5-mL of methanol to produce methanol-HCl to be used in the reduction reaction because it consumes hydrogen. The reaction was run dry with argon because acetyl chloride reacts violently with water. In 3-mL of methanol was dissolved 0.350 g (2.1 mmol) of the bicyclic **compound 1.** A slight excess of $NaCNBH_3$ (0.157 g, 2.5 mmol) was dissolved in the mixture. Several drops of methyl orange were added as a pH indicator.

Drops of methanolic-HCl were added to preserve acidity below 3.1 pH.[26] Methyl orange appears red below 3.1 and yellow above 4.4 pH. The object was to maintain the red color. This afforded a mechanism of measuring the reaction progress: initially, the color changed rapidly back to yellow, necessitating additional acid and indicating the consumption of hydrogen. After approximately an hour, the rate of color change slowed significantly indicating the reaction was proceeding to completion. The reaction was finished with a slightly red tint so as to ensure the reaction was complete.

Excess methanol was removed by rotary evaporation. The dark brown residue resulting was taken up in 5-mL of water and saturated with HCl. The mixture was extracted with ether (3 increments of 10-mL). The combined organic portions were dried over $MgSO_4$, filtered, and evaporated leaving a crude yield of 42%.

C. Reduction with Hindered Selectride Reagents:

Glassware was dried for 24 hours at 120°C. Using 5-mL of anhydrous THF, 0.480 g (2.86 mmol) of bicyclic **compound 1** was dissolved and stirred with a magnetic stir bar. The solution was purged and flushed with argon. Then, it was placed in a Dewar flask, where the temperature

was taken down to -78°C. Through an airtight syringe and needle, three equivalents of K-selectride (1M, 8.6-mL) were added. The mixture was allowed to react for 1 hour at -78°C, after which time a dark orange colored developed. The solution was warmed to 0°C in a Dewar flask. An oxidative work-up was begun with the addition of 10% sodium hydroxide and 30% hydrogen peroxide in the volumes of 7-mL and 5-mL, respectively.[27] The work-up was allowed to stir overnight. The aqueous layer was then extracted three times with 20-mL of dichloromethane. The combined organic layers were washed with the following: water in two 20-mL increments, 20-mL of saturated $NaHCO_3$, and 10-mL of NaCl. The organic component was then dried over magnesium sulfate, filtered, and evaporated leaving yellowish oil.

This procedure left an unsatisfactory product. In the new experiment, 0.150 g of bicyclic **compound 1** (1 mmol) was dissolved in 7-mL of dry THF and cooled to -78°C. Two equivalents of the hindered reduction reagents were used. At a concentration of 1M, this means 2-mL (2 mmol) of each was added. The selectride reduction reagents were added via an automated syringe plunger over the course of five minutes to ensure homogeneous and controlled addition. In this case, the reaction was allowed to proceed at -78°C for approximately two-and-a-half hours. Reaction completion was demonstrated by TLC (3:2 hexane to ethyl acetate eluting mixture) through the disappearance of the bicyclic peak.

A new work-up was selected to minimize the vigorous oxidative conditions with the intent of using milder conditions. It was then quenched with 5-mL of saturated NH_4Cl, washed with 10-mL of water, and the aqueous layer was extracted with dichloromethane (3 x 10-mL).[28] The combined organic layer was washed with saturated NaCl and dried over $MgSO_4$. After evaporation, the residue was purified by column chromatography to remove the alkylborane byproducts. The mixture was first eluted with 200-mL of 90% hexane and 10% ethyl acetate,

followed by 50-mL of 60% hexane/40% ethyl acetate, then 50-mL of 40% hexane/60% ethyl acetate, and lastly with 50-mL of ethyl acetate to clear out any remaining constituents.

Because the pure single isomeric product synthesized with K-selectride was not found in the chromatography collections until the latter fractions, a more polar solvent elution sequence was designed for the L-selectride reaction. Using the 10:1 silica to crude product mass rule-of-thumb, about 50 grams of silica were used in the preparation of the column because the crude mass was 5.2 grams. For the L-selectride reaction, the following chromatography solvent conditions were employed: 100-mL of 1% ethyl acetate in dichloromethane; 100-mL of 25% ethyl acetate in dichloromethane; 75-mL of 50% ethyl acetate and 50% dichloromethane; 75-mL of 75% ethyl acetate and 25% dichloromethane; 75-mL of 25% methanol and 75% dichloromethane; and concluded with 50-mL of straight methanol. The chromatography was monitored by TLC with an eluting mixture of 25% ethyl acetate and 75% dichloromethane. The yield was nearly quantitative – 98.8%.

IX. Grignard Addition:

After drying of glassware, a 100-mL volumetric flask was fitted with a magnetic stir bar and 0.5 g (2.97 mmol) of bicyclic was placed inside. The flask was flushed with argon and cooled to the appropriate temperature (0°C, -15°C, and -78°C) or allowed to remain at room temperature. From the solvent manifold, anhydrous THF was obtained and put under argon. A volume of 15-mL of THF was injected via syringe into the flask as the reaction solvent. The dissolved bicyclic resulted in an orange tint. Methyl magnesium bromide (CH_3MgBr) was allowed to warm to room temperature and flushed with argon. Using an syringe pump, 1.00-mL of CH_3MgBr (3M, 3 mmol) was added to the flask at a rate of 4-mL/hr, or 15 minutes for

complete transfer. After two hours of reaction, the mixture was quenched with 75-mL of ammonium chloride (NH_4Cl) to kill any remaining Grignard reagent. The organic layer was separated and the aqueous layer was extracted with anhydrous THF (3 x 15-mL). The combined organic layers were evaporated, dissolved in diethyl ether, dried with $MgSO_4$, filtered, and evaporated to again remove solvent.

In the case of the deuterated work-up, D_2O was added where ammonium chloride was previously and directly put on the rotary evaporator. The mixture was not extracted or dried because D_2O does not appear in ^{1}H-NMR experiments.

APPENDIX B - STEREOCENTERS:

Stereocenters are often found in biologically active compounds. If four single bonds are formed at a single carbon and the atoms attached are all chemically dissimilar, a stereocenter, or chiral center, has resulted. Two different arrangements of these groups, which are mirror images, are possible. These non-superimposable mirror image compounds are known as enantiomers, shown in **Figure 33**.[7]

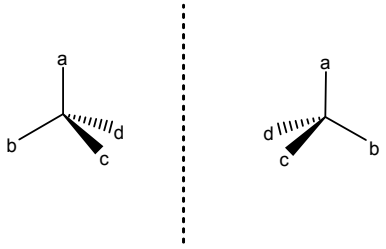
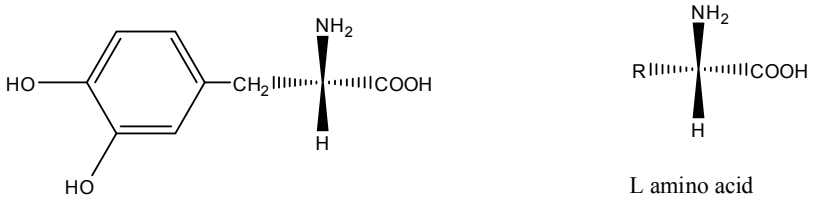


Figure 33: Enantiomers

Controlling this stereochemistry is of great importance because the biological world usually relies on only one enantiomeric version of the two. Consider amino acids, which exist solely as the (L) stereoisomer of the respective molecule. Another example pertains to L-Dopa, which is used to treat Parkinson's by conversion to dopamine in the brain. Its enantiomeric cousin, D-Dopa, cannot achieve this desired activity.[29] The active form, along with a typical amino acid, is displayed in **Figure 34.** Not only is nature usually selective for one enantiomer, but the second isomer might actually have toxic or dangerous side effects if combined with the active molecule. For this reason, the FDA often requires that a new drug consist of only one stereoisomer or proof that the other is not harmful when combined in a racemic mixture, or a 1:1 mixture of enantiomers.



L-Dopa, an anti-Parkinson's drug

L amino acid

Figure 34: L-Dopa and a Typical L-Amino Acid

APPENDIX C – MECHANISMS:

I. Mechanism of the Horner-Wadsworth-Emmons Reaction:

Once the triethyl phosphonoacetate reagent (**compound B**) is deprotonated, the nucleophilic carbon attacks the electron-deficient carbon of the ketone carbonyl. This causes a negative charge to be transferred to the oxygen and then formation of a new carbon-carbon bond shown in compound **C**. The oxygen then bonds to the phosphorus atom in a Lewis acid-base reaction, resulting in the four-membered intermediate highlighted in compound **D**. This intermediate then fragments to give the new alkene and a dialkylphosphate salt byproduct.

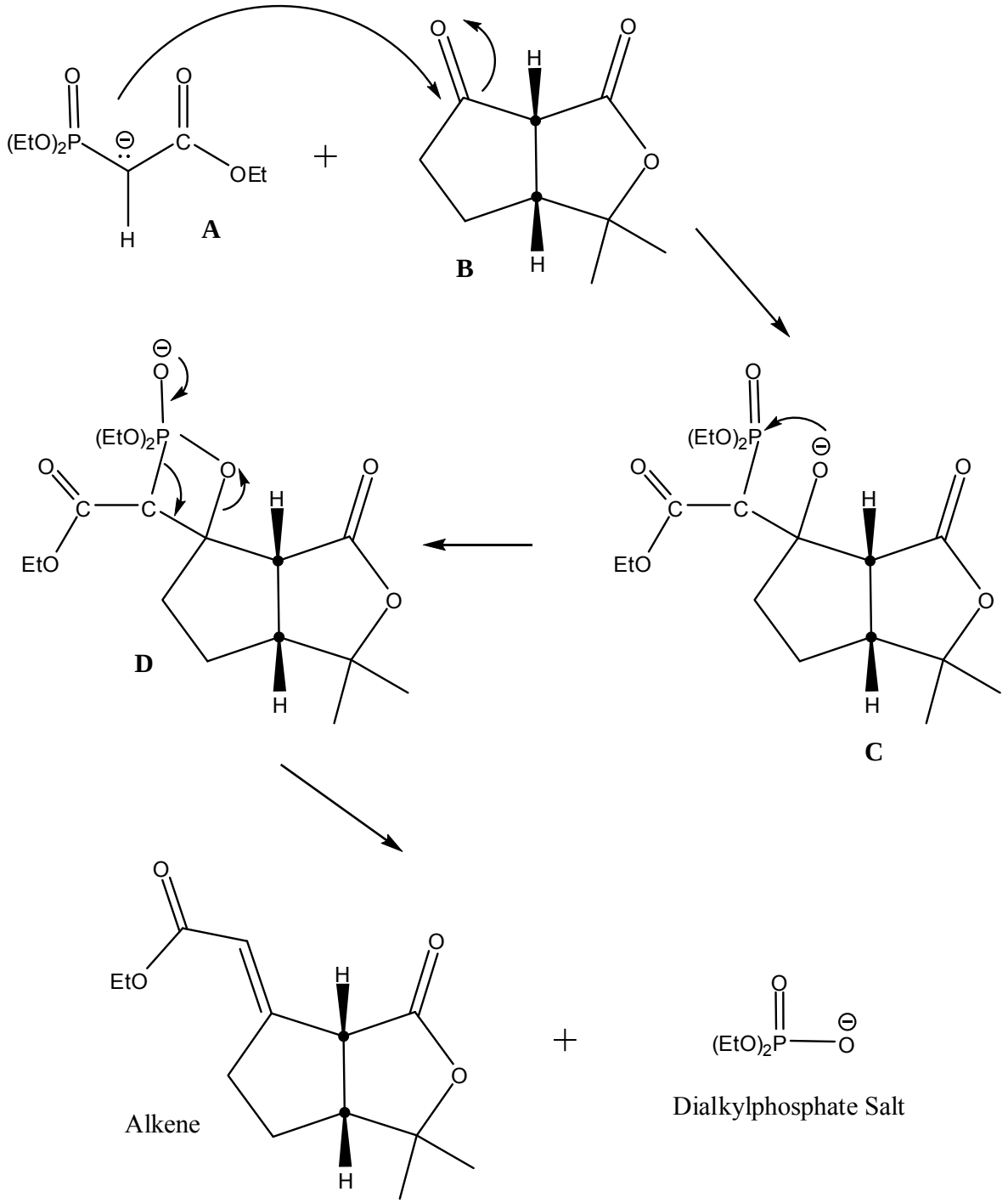


Figure 35: Mechanism of the HWE Reaction

II. Mechanism of the Wittig Reaction:

When the carbon is deprotonated to create the ylide reagent, the reaction begins with the negative carbon attacking the electrophilic carbon center of the carbonyl, shown in **step A**. The intermediate formed is known as a betaine.[30] Acting as a Lewis base, the oxygen then forms a covalent bond to the phosphorus atom in **step B**. The new four-membered intermediate is called an oxaphosphetane.[31] When heated, this ring structure spontaneously collapses leaving the product alkene and the triphenylphosphine oxide byproduct as shown in **step C**.

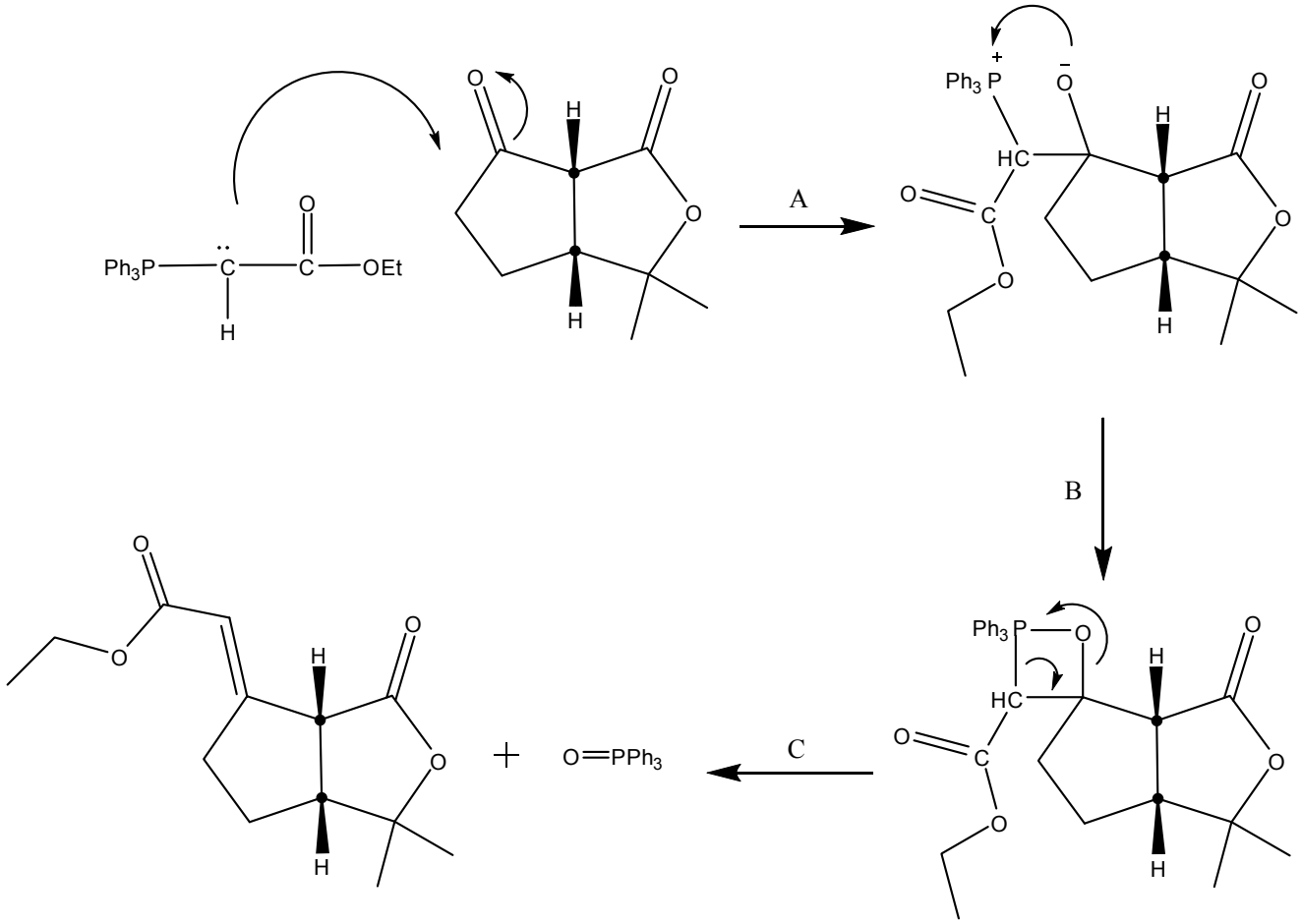


Figure 36: Mechanism of the Wittig Reaction

III. Conjugate Addition Mechanism

For years, the mechanism by which cuprate reagents actually achieved conjugate addition has been debated. Recently, however, the debate was settled by researchers at the University of North Carolina, Charlotte who confirmed the existence of a predicted copper (III) intermediate by rapid-injection NMR spectroscopy.[32] Shown below in **Figure 37** is the reaction of 2-cyclohexenone with lithium dimethylcuprate, which represents a general 1,4-conjugate addition mechanism.

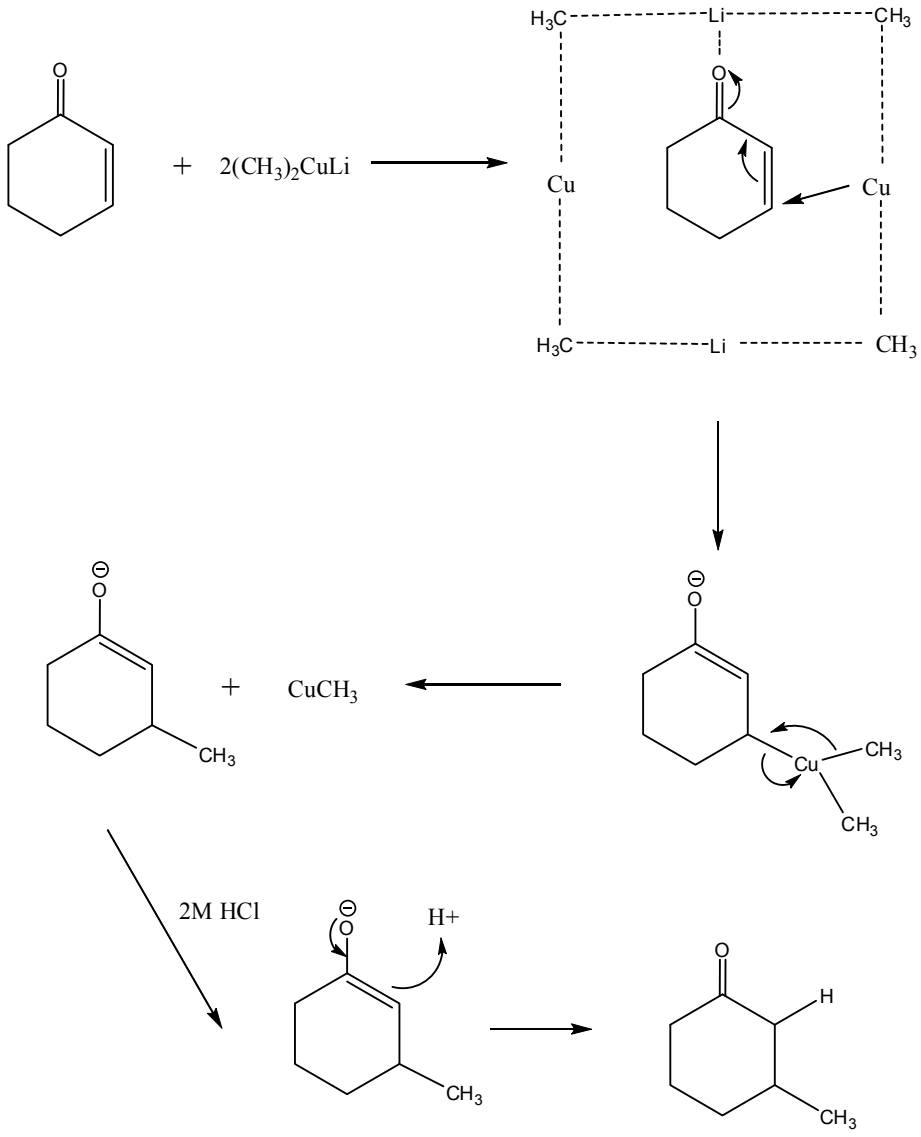


Figure 37: Mechanism of Conjugate Addition with Lithium Dimethylcuprate

IV. Proposed Cuprate Addition Mechanisms

Although unprecedented, it was concluded from NMR analysis that the cuprate actually attacked the carbonyl of the lactone ring. The proposed mechanism discussed in the results section is shown in **Figure 38** below.

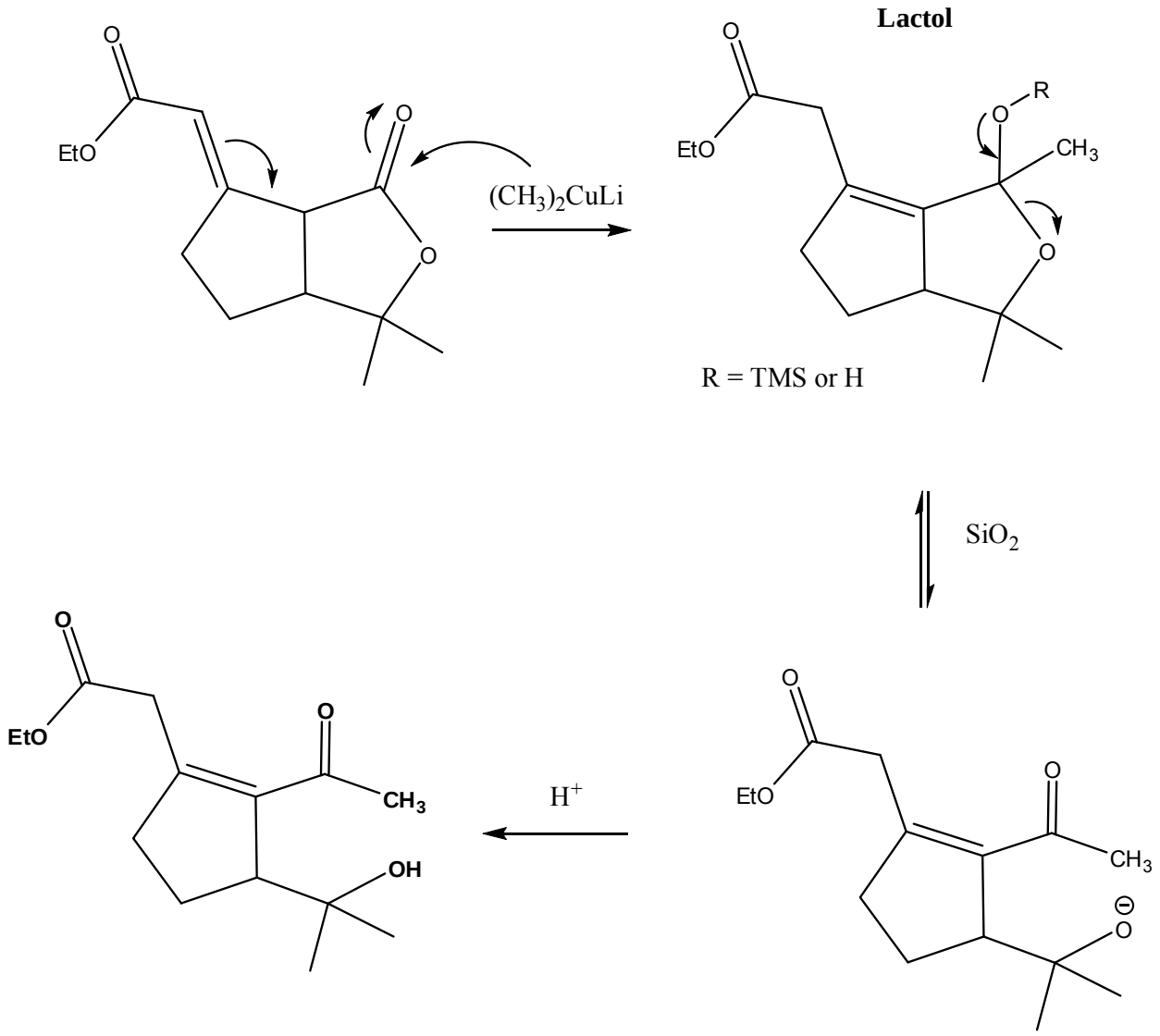


Figure 38: Proposed Lactone Addition Mechanism

APPENDIX D – NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY:

The standard for compound characterization in organic chemistry is spectroscopy. Spectroscopy is the study of how electromagnetic radiation interacts with matter. The material is subjected to some form of energy and the subsequent emission or absorption is measured and plotted in a functional manner. To characterize the products of synthesis in this project, two forms of spectroscopy were primarily utilized: nuclear magnetic resonance and gas chromatography/mass spectrometry.

Nuclear magnetic resonance utilizes the magnetic spin properties of atomic nuclei. Hydrogen, for example, has two possible spin values that are degenerate (equal in energy) under normal conditions. However, when placed in the presence of an external magnetic field, the spin states are no longer equal.[33] The spin states are alternatively either aligned with or against the magnetic field, producing an energy differential that can be measured and quantified. This energy difference, ΔE highlighted in **Equation 1**, is proportional to the strength of the external magnetic field, B_o, and a quantity known as the magnetogyric ratio, γ, which is characteristic of the isotope being observed. The term, h, is Planck's constant and has a value of 6.626×10^{-34} J*s.

$$\Delta E = h \gamma B_o/2\pi \qquad \textbf{Equation 1}$$

Once this energy difference is determined, the sample is bombarded with radiation at a frequency (**Equation 2**) with equal energy. Normally, the frequencies pertinent for NMR fall in the radio portion of the electromagnetic spectrum. This energy application causes resonance of the nuclei and a corresponding transition to the higher energy state. When the nuclei decay to the more predominant lower energy level, radio frequencies are released that generate a

complicated signal. Using Fourier transformation, a computer processes these free induction decay signals and displays the data in a readable format. The whole process normally is completed in a matter of seconds.

$$\nu = \Delta E / h \qquad \textbf{Equation 2}$$

Because the local electronic environments of hydrogens in molecules differ, different signals will be produced depending on how the hydrogen is bonded. As electrons circulate, they generate their own new magnetic fields that interact with the external magnetic field applied. Depending on the position in the molecule, hydrogens require varying absorption frequencies that result in characteristic chemical shifts that can be imaged. In order to normalize chemical shifts of different spectrometers, tetramethylsilane (TMS) is typically employed as an internal standard. The high electron density of TMS means a large magnetic field is induced, which causes diamagnetic shielding and a shift far upfield.[34] Thus, TMS generally appears at 0 ppm with the majority of organic molecules falling more downfield. The structure of TMS and a stereotypical peak representation are given in **Figure 39**. Downfield shifts are the result of inductive effects specific to compounds and functional groups. Electron withdrawing groups pull electron density from hydrogens, deshielding them and shifting the peaks downfield. Groups of low electronegativity tend to donate electron density, producing upfield shifts. Functional groups which donate electron density through π bonding, which is an electron association through space, also contribute to upfield shifting.

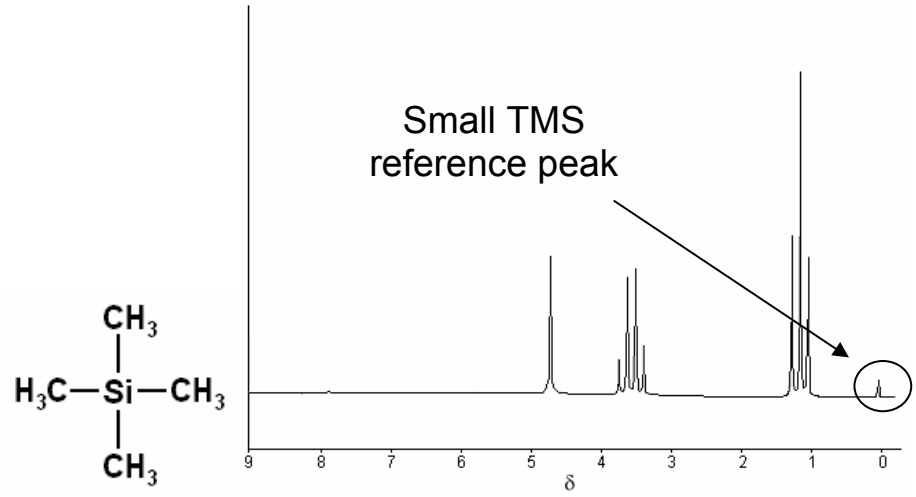


Figure 39: Structure of TMS and Typical NMR Position

In addition to the force of the external magnet, hydrogens are influenced by the relatively smaller magnetic fields generated by nearby hydrogens. These interactions split the absorption peaks of hydrogens in predictable fashion, an effect known as spin-spin coupling.[35] In fact, the multiplicity (the number of splits within a single peak) combined with the previously discussed shifting allows for the assignment of hydrogens within molecules based on knowledge of neighboring hydrogens. Basically, there are two different splitting possibilities relevant to the project. The first concerns the splitting of absorption peaks by equivalent hydrogens. In general terms, hydrogens coupled to *n* equivalent hydrogens will split into *n* + *1* peaks.[35] Coupling refers to neighboring hydrogens that cause splitting in the signal. The separation between two peaks in a signal is known as the coupling constant, *J*, and is measured in hertz.[36] The coupling constant is independent of the operating frequency of any particular instrument.

To illustrate, consider the Compound ethyl iodide (CH_3CH_2I) shown in **Figure 40** below. The hydrogens bonded to the right-hand carbon generate a signal at 3.2 ppm. The downfield shift is due to iodine, which is electron withdrawing. This signal will be split into 4 peaks because there are 3 hydrogens bonded to the neighboring carbon. The hydrogens on the terminal

carbon will cause a signal at 2.7 ppm. Similarly, this signal will be split into 3 peaks because there are 2 hydrogens bonded to the right-hand carbon. These splitting patterns are typical of vicinal hydrogens and are often referred to as three-bond coupling. The terminal methyl group, which is relatively nonpolar, will fall upfield. These trends are evidenced in the actual ^{1}H-NMR peak for ethyl iodide shown in **Figure 41.**

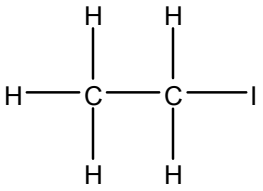


Figure 40: Structure of Ethyl Iodide

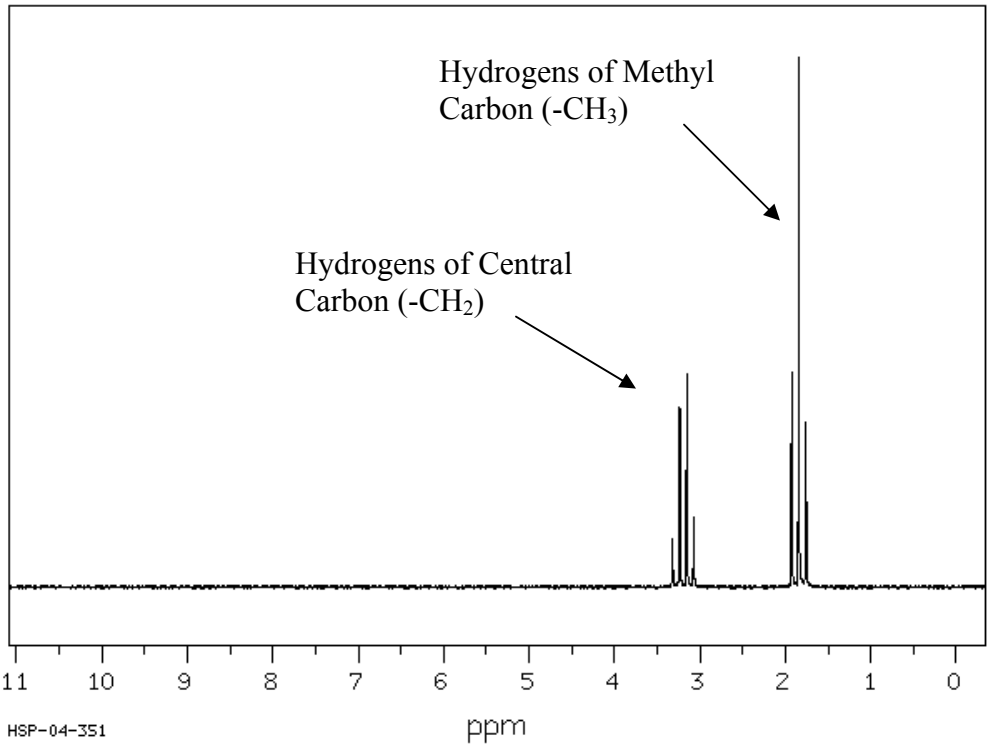


Figure 41: ^{1}H-NMR Spectrum of Ethyl Iodide

Secondly, sometimes hydrogens bonded to the same carbon, or geminal hydrogens, do not split identically as was the case in the previous example. With ethyl iodide shown above, the three hydrogens bonded to the methyl group and the two hydrogens of the $–CH_2$ group are degenerate and give single peaks at characteristic chemical shifts. This degeneracy is caused by unrestricted rotation of the C-C single bond in the molecule.

With compounds that are restricted in their bond rotation, independent peaks for hydrogens bonded to the same carbon occur. The rigidity causes different chemical environment in space and the splitting patterns for the hydrogens will result in separate peaks because of the influence of the unique chemical groups surrounding the nuclei. In this case, the coupling constants, *J*, will be different for each association. For example, consider the spectrum of 2-amino-1-butanol shown in **Figure 42**. The hydrogens bonded to the carbon C-1, attached to the hydroxyl group fit the non-equivalence criterion. The peak of the hydrogen pointed away from the amino group, H_b, appears as a "doublet of doublets" at approximately 3.2 ppm, caused by the unequal coupling influence of both the geminal hydrogen, H_a, bonded to the same carbon and the vicinal hydrogen, H_c, bonded to the middle adjacent carbon. The peak of the hydrogen closest to the amino group, H_a, appears as another doublet of doublets centered around 3.5 ppm, caused by the combined coupling effects of the geminal hydrogen, H_b, the vicinal hydrogen, H_c. The differences in the splitting patterns for each signal can be attributed to different splitting constants, *J*, for each proton interaction. This complex splitting is entirely different from the simpler splitting observed with ethyl iodide and is indicative of the type of splitting which has been observed for the bicyclic starting Compound and the subsequent synthesis products. Since bicyclic Compounds are so rigid, two hydrogens bonded to the same carbon will give completely separate peaks.

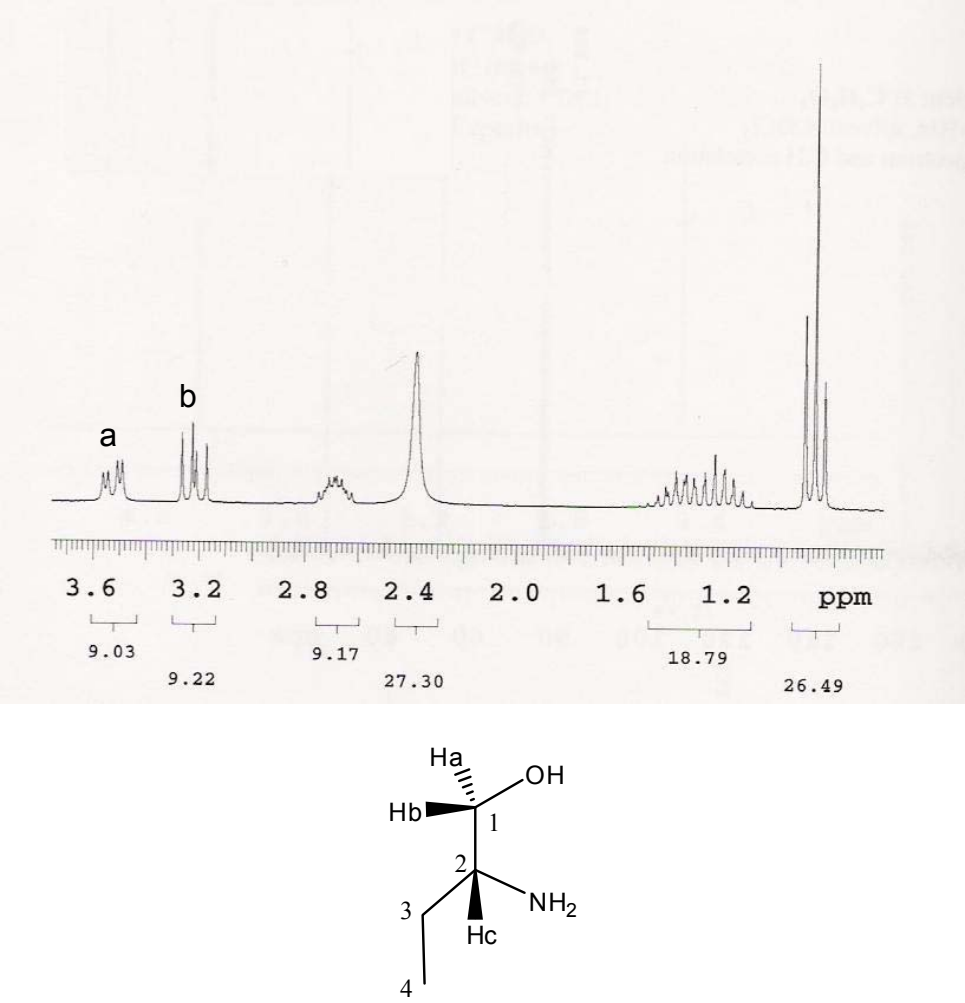


Figure 42: ^{1}H-NMR Spectrum and Structure of 2-amino-1-butanol

^{13}C-NMR:

Thus far, only the ^{1}H isotope of hydrogen has been considered. Usually, only isotopes with odd atomic numbers produce spectra because of the influence of a parameter known as the spin quantum number, *I,* which impacts magnetic spin properties.[37] For the purpose of structure elucidation, the most valuable nuclei are those that exhibit a spin quantum number of ½. The ^{1}H isotope is an example of a nucleus with this value. There is another isotope of considerable worth also holding this value: ^{13}C. The characteristics of carbon-13 are considerably different

and complement this hydrogen data nicely. In particular, the peak for each carbon appears as a singlet with peaks spread across a much broader shift range.[38] This broader chemical shifting means carbon peaks rarely overlap and each carbon within a molecule typically gives a distinct peak. Consider the carbon-13 spectrum for ethyl iodide, which was previously used to illustrate a simple ^{1}H-NMR spectrum, shown in **Figure 43**. Here, only two peaks appear representing the two carbons in the molecule. The peak at about 20 ppm is attributed to the carbon bonded to iodine, which falls farthest downfield because the same electronegativity principles articulated before. The alkyl peak appears at approximately 0 ppm due to its non-polar nature. Since carbon atoms are involved in the skeleton of organic structures, ^{13}C spectra are very useful. One complication of carbon NMR is that most carbons in nature (>99%) are ^{12}C, which generates no NMR spectra because of its even atomic number. ^{13}C atoms are NMR active but comprise only ~1% of all carbons. Therefore, acquiring ^{13}C spectra is time-consuming and generally gives poor signal-to-noise ratios compared to ^{1}H-NMR.

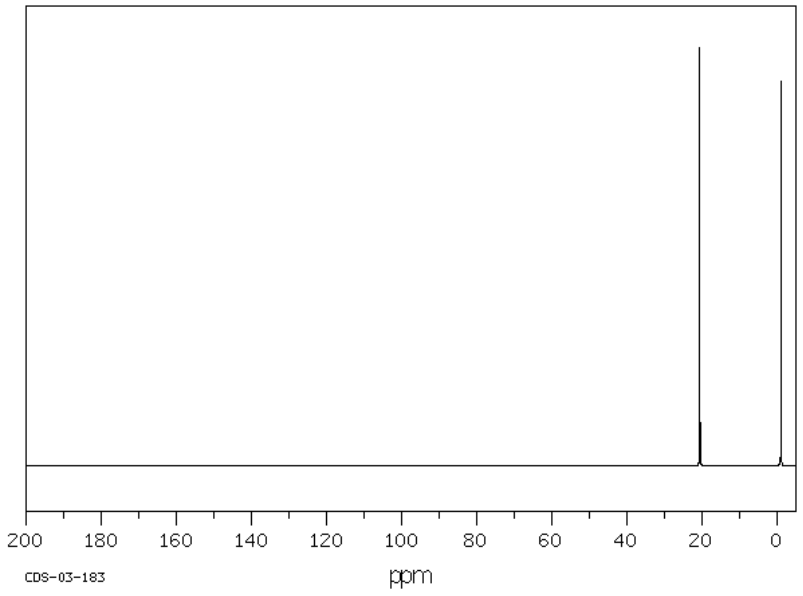


Figure 43: ^{13}C-NMR of Ethyl Iodide

Compound 2, the product of the first synthetic reaction, consisting of 13 carbons yielded 13 distinct peaks in the ^{13}C-NMR spectrum collected. The structure of the Compound is shown in **Figure 44**.

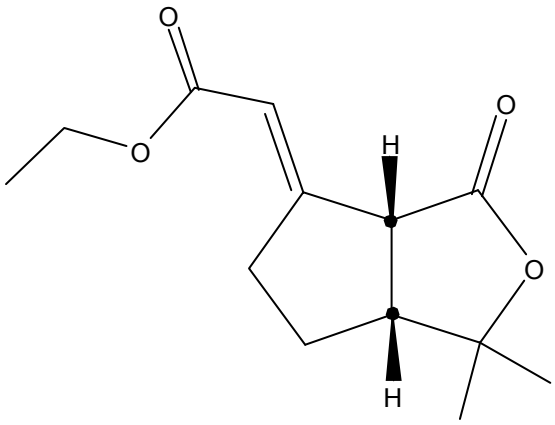


Figure 44: Compound 2 – The Unsaturated Ester

Fortunately for analytical purposes, the chemical shifting caused by the electronegativity of neighboring atoms evidenced in ^{13}C-NMR spectroscopy mirrors that of ^{1}H-NMR, with the exception that the peaks are much more spread out and splitting is not seen as in proton experiments.[38] Carbonyl carbons (C=O) of esters fall far downfield in the 160-185 ppm range, whereas alkyl carbons fall upfield in the 0-50 ppm range. In the case of the α,β-unsaturated ester above, the carbonyl carbons fell at 165 ppm and 175 ppm, as expected. Alkene carbons typically fall in the 100-170 ppm range.[38] The two carbons of the alkene bond appeared at approximately 115 ppm and 160 ppm, in accordance with empirical precedence. Carbons singly bonded to oxygen as in ethers or alcohols typically fall in the 50-90 ppm range. Again, as predicted, the carbon bonded to the oxygen of the ester appeared at about 85 ppm. Thus, the remaining eight carbons representing alkyl groups were assigned in the expected upfield region. These delineations can all be seen in **Figure 45**, the actual ^{13}C-NMR spectrum. The large signals at ~77 ppm are due to the solvent, $CDCl_3$, which is necessary for acquiring the spectrum.

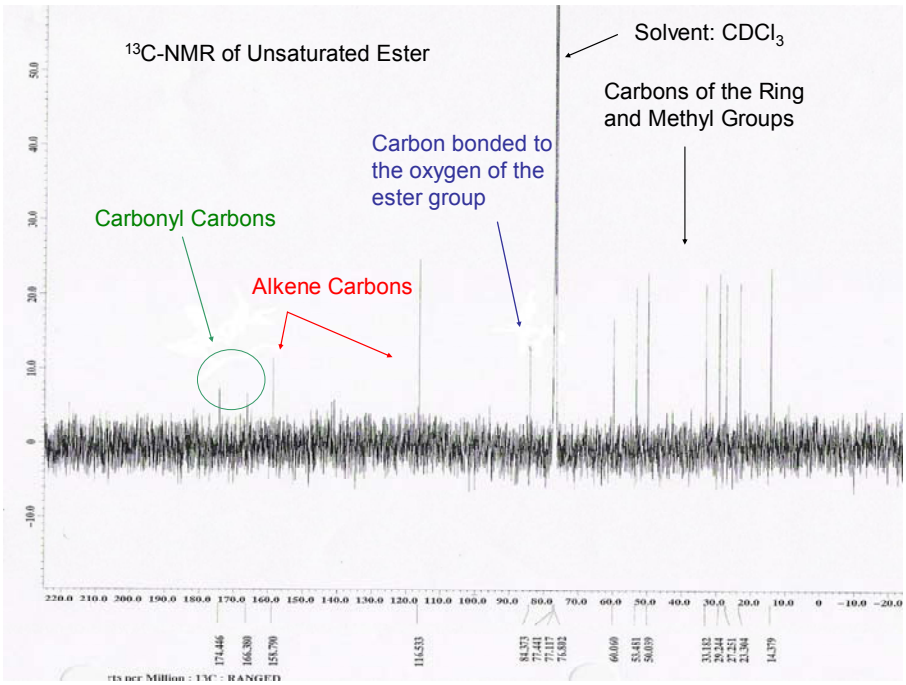


Figure 45: ^{13}C-NMR spectrum of Compound 2

This form of carbon-13 NMR also helped to confirm whether the catalytic hydrogenation and reduction reactions were successful. In the case of the tertiary stereocenter synthesized through hydrogenation, thirteen carbon peaks should still appear but the downfield shifted peaks of the alkene shown in **Figure 45** should disappear with two new peaks emerging in the alkyl portion of the spectrum. This was precisely the case seen, as evidenced by the ^{13}C-NMR of the hydrogenation product (**compound 3)** obtained with the platinum catalyst shown in **Figure 46.** Here, the two alkene peaks are gone with 10 peaks appearing in the alkyl region of the spectrum vice only eight for the alkene. The two peaks on either side of 130 ppm should be ignored, as they are from residual phosphine oxide byproducts of the earlier Wittig reaction. Similarly, when the ketone is reduced in the borohydride reactions, the carbonyl peak that would otherwise appear in the 160-180 ppm range disappears.

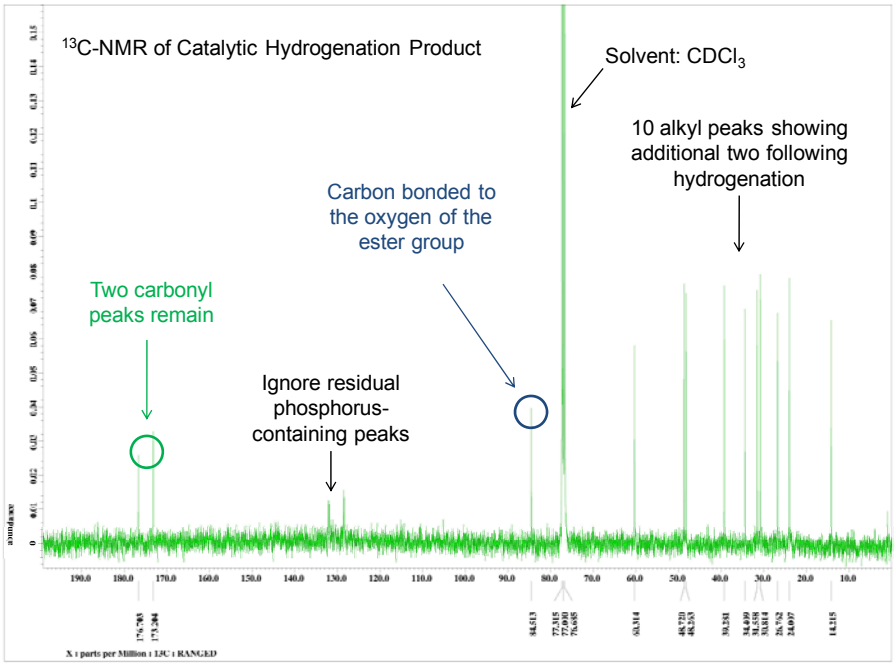


Figure 46: ^{13}C-NMR spectrum of Compound 3

Two-dimensional NMR:

To this point, only one-dimensional NMR techniques have been presented. One-dimensional NMR consists of a single pulse of radio waves exciting the nuclei. The experiment may be repeated and the signals averaged to generate the spectrum. Of great use to the organic chemist are techniques known as two-dimensional NMR, which allow for nearly complete structural determination. They are especially useful for completely assigning NMR signals for structures that are predicted or already known, as is the case with this project.[39] Essentially the procedure consists of a series of one-dimensional techniques combined. Radio pulses are generated with a predetermined time delay between the signals. Then, the excited nuclei rotate in the magnetic field for a set evolution time. Following the last pulse, the frequencies of

relaxations are measured as in one-dimensional NMR. By varying the evolution time, the one-dimensional signals from each pulse sequence can be summed to form the two-dimensional spectrum.[39]

Homonuclear two-dimensional techniques consist of correlating the one-dimensional scans of a single isotope of the same element. Where nuclei couple, a three-dimensional peak is generated which is represented on paper by a contour plot, similar to the way elevation is indicated on maps. The most useful homonuclear technique for this project was ^{1}H-^{1}H COSY, or ^{1}H correlation spectroscopy. The diagonal line of spots is the contour plot of the one-dimensional spectrum. Hydrogens that couple to each other are imaged, as indicated by black contour spots appearing off the diagonal. The spots appear at the intersection of vertical and horizontal lines extended from hydrogen peaks on the same respective axes. The ^{1}H-^{1}H COSY plot for the unsaturated ester **compound 2** is displayed in **Figure 47**. The one-dimensional ^{1}H-NMR spectrum for the compound is plotted on both the *x*- and - and *y*-axes.

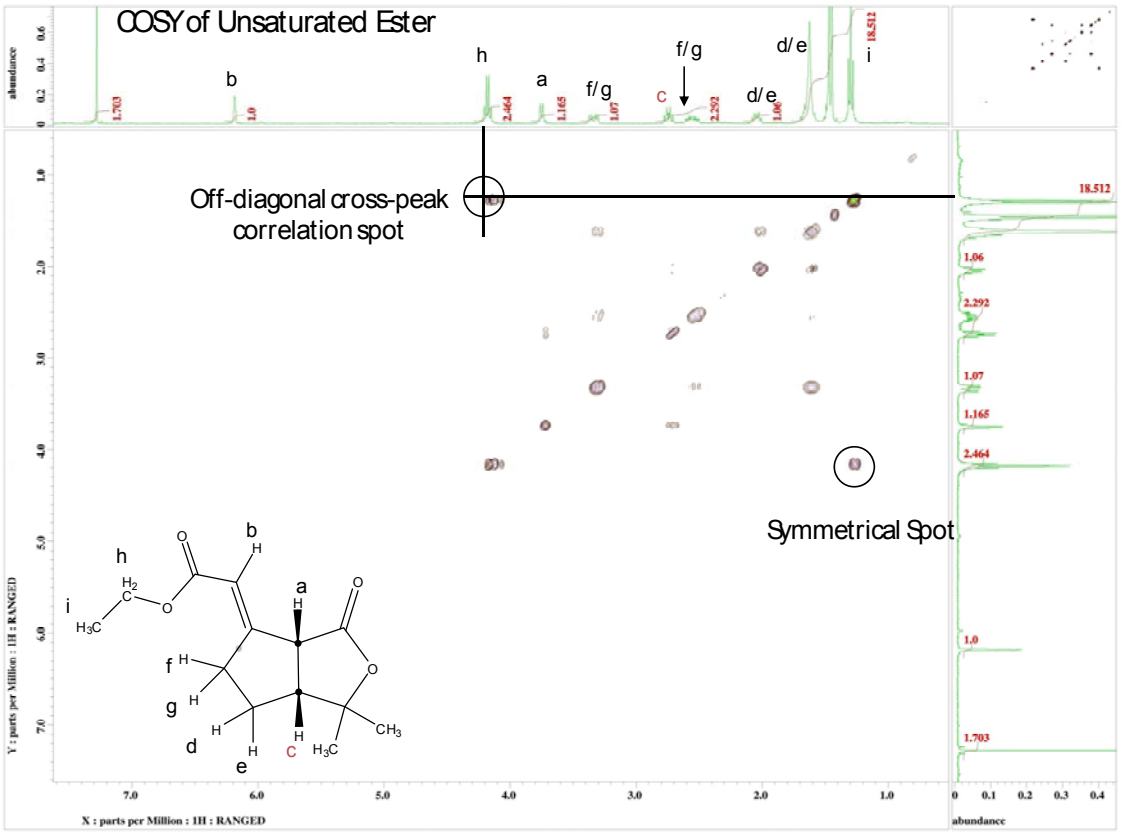


Figure 47: ^{1}H-^{1}H COSY Spectrum of Compound 2

The spectrum is interpreted by first reading the diagonal. The peaks off the diagonal, known as cross-peaks, at first glance seem less ordered. However, each cross-peak has a corresponding symmetrical peak on the opposite side of the diagonal. This symmetry is indicative of hydrogens that are spin-spin coupled. The hydrogens labeled *h* bonded to the methylene group in the Figure above are illustrative of the overall principle. The only spot off the diagonal correlates to the peak of the methyl hydrogens at ~1.2 ppm in the one-dimensional ^{1}H-NMR spectrum; the spot also has a symmetrical spot appearing across the diagonal. This data confirms the expectation that the methylene group would only couple with the methyl group, which is the only group it is bonded to with hydrogens that could cause splitting. Similar

reasoning was applied to the assignments for the remainder of the hydrogens labeled in the Figure.

Heteronuclear correlation experiments where different isotopes or different elements are plotted and compared are also of great benefit in structural elucidation. For example, HMQC allows the chemist to correlate hydrogen and carbon connectivity by correlating the ^{1}H-NMR spectrum of a Compound to its corresponding ^{13}C-NMR spectrum. As in COSY, two lines drawn at right angles from a ^{1}H peak and a ^{13}C which intersect at a cross-peak (a black contour spot) indicate to which carbon the particular hydrogen of interest is bonded.[40] The HMQC spectrum obtained for the unsaturated ester **compound 2** provided additional spectroscopic data for more thorough identification of those hydrogens which remained ambiguous from the previous experiments. Moreover, the HMQC spectrum provided evidence that the new alkene hydrogen was indeed bound to the corresponding alkene carbon, as shown below. Also highlighted is the intersection of the ^{1}H-NMR peak and the ^{13}C-NMR peak of the methylene hydrogens and carbon, respectively. The spectrum itself is shown in **Figure 48.**

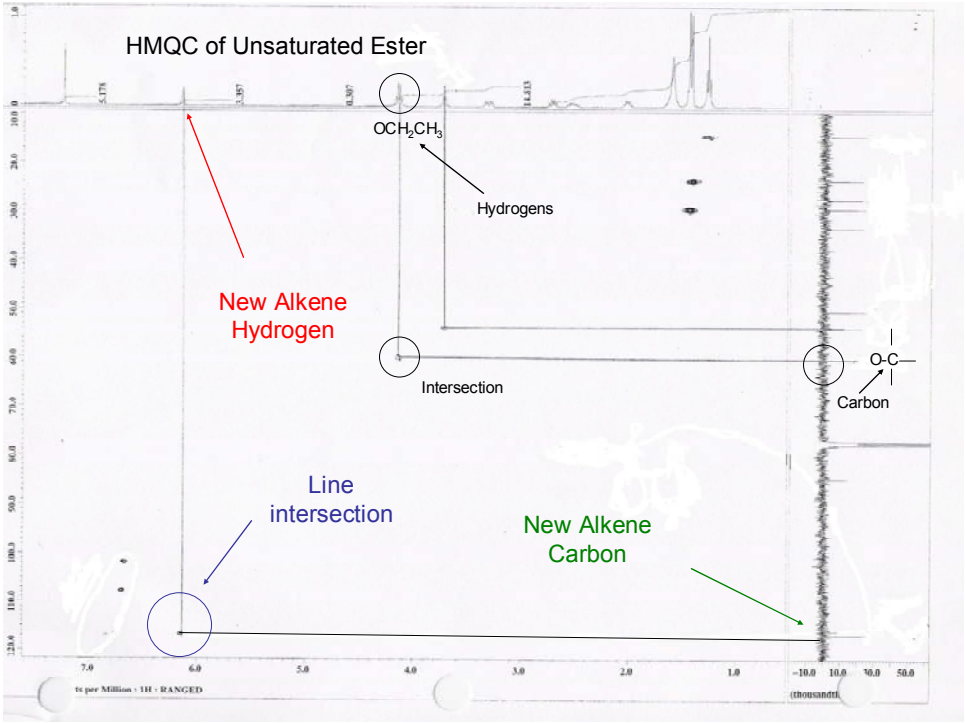


Figure 48: HMQC Spectrum of Compound 2

Two-dimensional techniques are also what allowed the hydrogens of the hindered reduction product and the hydrogens of the catalytic hydrogenation to be assigned. For the catalytic hydrogenation product (**compound 3)**, three experiments were combined to deduce the hydrogen assignments. The first two, ^{1}H-^{1}H COSY and HMQC, have already been introduced. The third, HMBC, stands for Heteronuclear Multiple Bond Correlation spectroscopy. In this experiment, correlations between spins that are two or three bonds apart appear as off-diagonal peaks.[41] On the horizontal axis, the ^{1}H-NMR is plotted with the ^{13}C-NMR plotted on the vertical axis as in HMQC. However, unlike HMQC, the correlation is not between hydrogens and carbons that are directly bonded but rather those that are several bonds apart. The bridgehead hydrogen for example was assigned as the peak at 3.30 ppm because it correlated to the lactone

carbonyl several bonds away. Similarly, the new hydrogen at the tertiary stereocenter correlated to the carbonyl of the ester. Both of these correlations are shown in **Figure 49** with the contours circled. Taking all of the spectra together is akin to completing a puzzle: taken alone no single spectra can provide all of the molecular information but together the entire structure can be assigned.

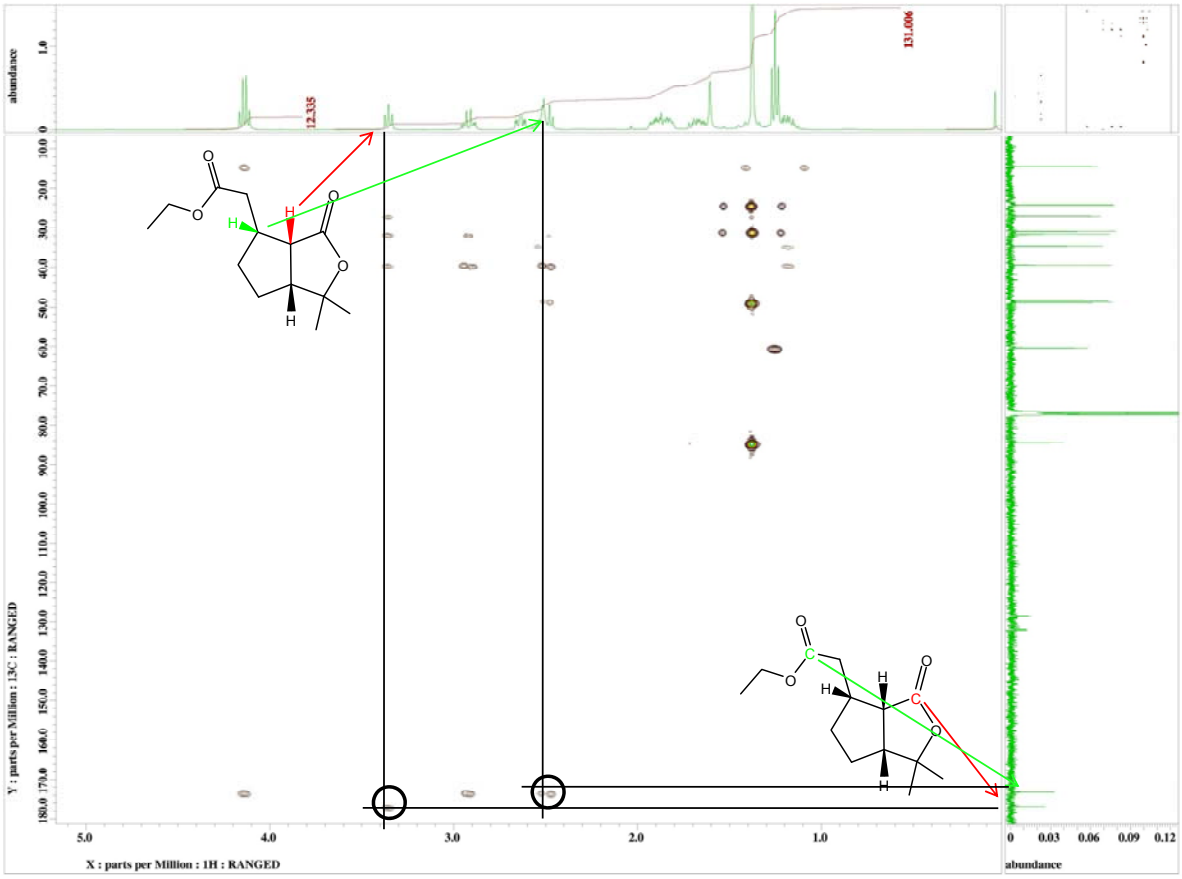


Figure 49: HMBC Spectrum for Compound 3

Nuclear Overhauser Effect Spectroscopy:

This is a technique that relies on through space interactions between nuclei close in proximity. The experiment is performed by first obtaining a ^{1}H-NMR spectrum of a Compound and then selecting a specific peak to directly irradiate with electromagnetic energy. When relaxing, a small portion of the energy will be lost through dipole-dipole mechanisms that exist through spatial distances, rather than the more powerful through bond means used by the other NMR experiments presented thus far.[42] Some of this energy will be absorbed by nuclei nearby. When the difference between the normal proton spectrum and the resulting nOe spectrum is taken, peaks due to atoms that were nearby in space to the nuclei irradiated will be enhanced from this additional energy. Those that were not close will not absorb any extra energy and will be absent from the spectrum because their peaks will cancel out when the difference is taken. The irradiated peak itself is inverted and phased so the enhancements can be more clearly interpreted. These peak enhancements are what afforded the assignment of stereochemistry necessary to confirm stereospecific synthesis. If peaks were enhanced in the bicyclic compounds, they must be on the same face of the molecule as the proton irradiated by virtue of the spatial nature of the technique.

With respect to the reduction products, the new hydrogen bonded to the carbon of the former ketone carbonyl was confirmed to be *cis* to the bridgehead hydrogens located at the intersection of the two rings. This was accomplished by conducting two separate nOe experiments. In the first, the top bridgehead hydrogen of **compound 4** was irradiated, causing the peaks of both the new hydrogen and the bottom bridgehead to be enhanced because of the close proximity in the three-dimensional space. The structure pictured in **Figure 50** below shows the irradiated bridgehead hydrogen colored in green and the enhanced hydrogens colored

in red. The enhanced peaks themselves are highlighted in the nOe spectrum presented in **Figure 51**. A zoomed ^{1}H-NMR spectrum of the Compound is attached whose peaks correspond to those in the nOe spectrum.

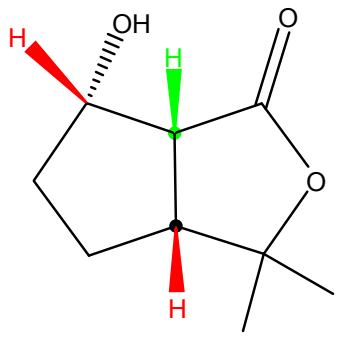


Figure 50: First Irradiated Hydrogen for nOe of Reduction Product

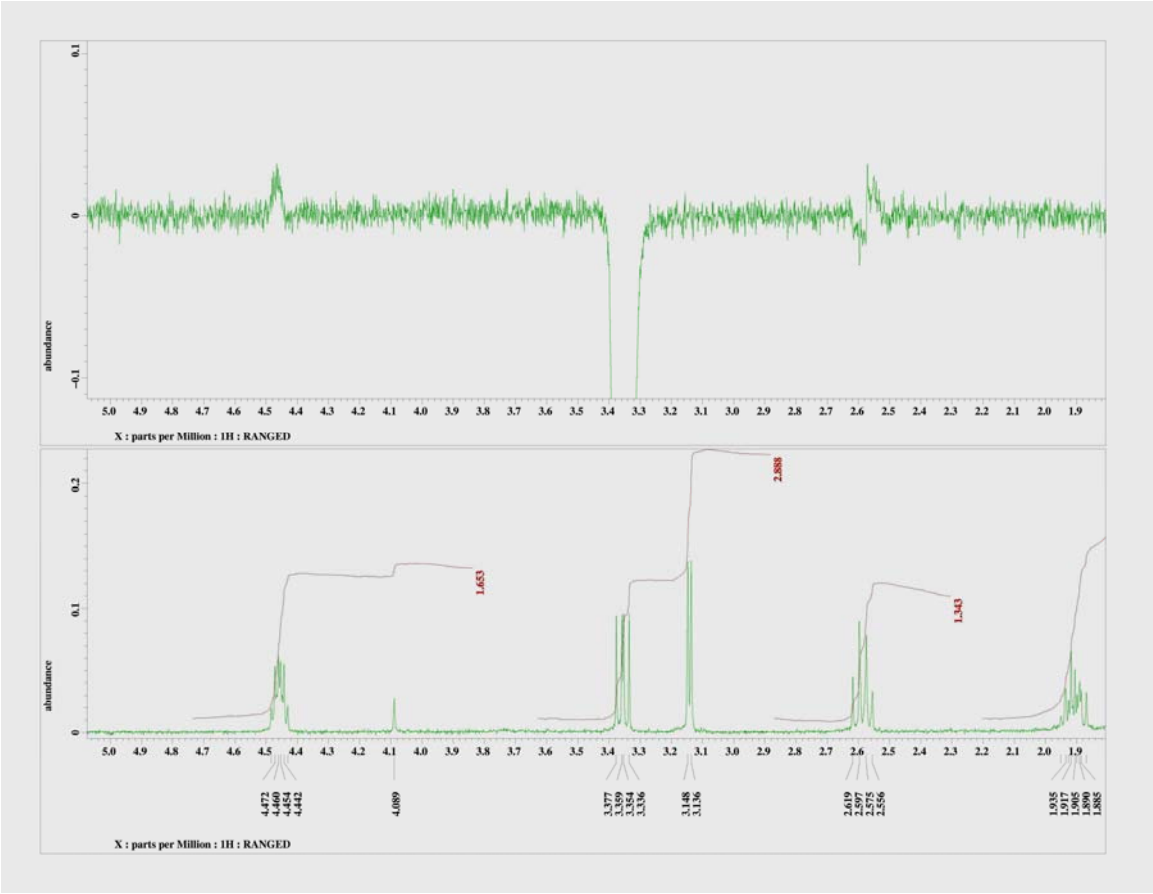


Figure 51: nOe Spectrum for the Reduction Product with Bridgehead Irradiated

To confirm this stereochemistry, the new hydrogen itself was irradiated causing the top bridgehead to be enhanced as well as the hydrogen bonded to the next carbon on the ring structure which projects on the same face of the molecule.

Similarly, the stereochemistry of the tertiary stereocenter in **compound 3** synthesized using the platinum catalyst was assigned using one-dimensional nOe. In this case, the top bridgehead hydrogen was again irradiated and enhancement observed for both the new hydrogen at the stereocenter and the bottom bridgehead hydrogen as shown in the spectrum presented in **Figure 52.** The Compound itself is shown in **Figure 53** with the irradiated hydrogen in red and the enhanced in green. Because the stereochemistry of the bridgehead hydrogens was known, the enhancement conclusively allowed the new hydrogen to be assigned as projecting on the same face of the molecule (*cis*) as the dual bridgehead hydrogens.

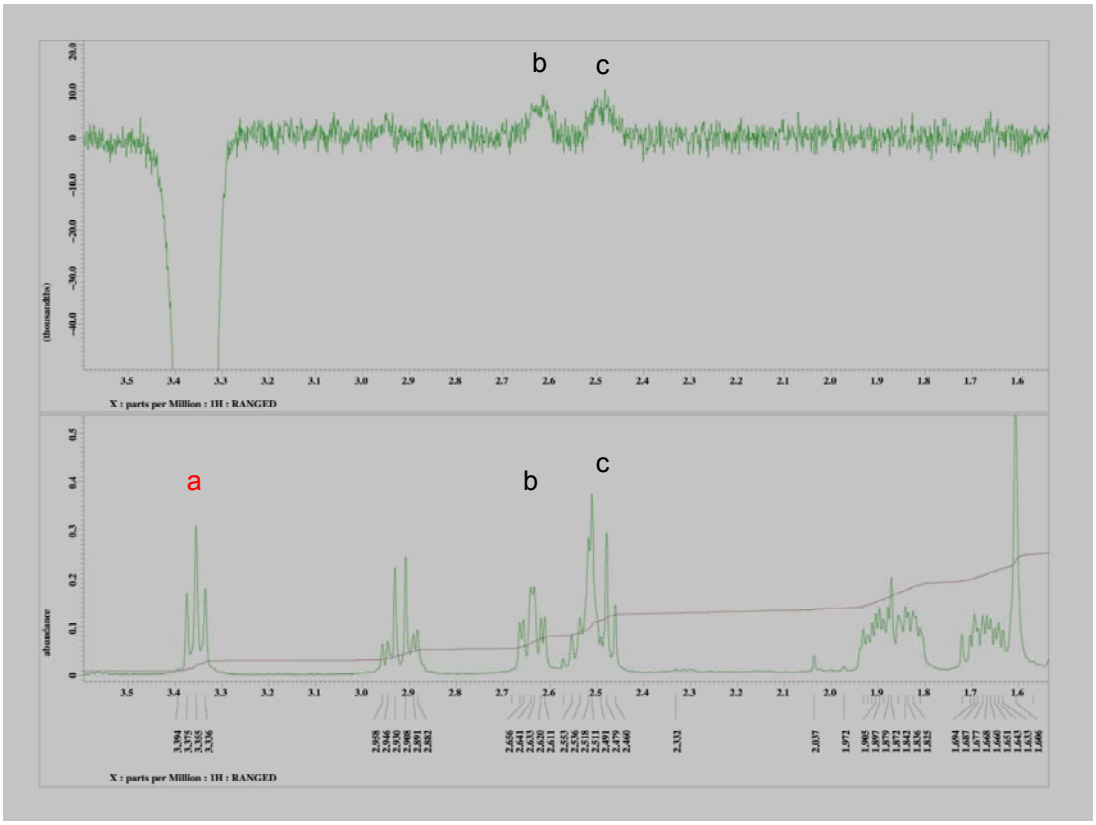


Figure 52: nOe Spectrum for the Pt Hydrogenation Product with Bridgehead Irradiated

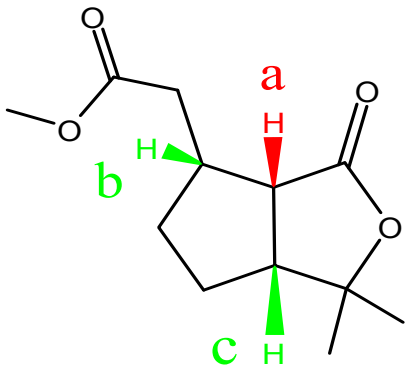


Figure 53: Irradiated and Enhanced Hydrogens for nOe of Hydrogenation Product

APPENDIX E – CHROMATOGRAPHY:

I. Thin Layer Chromatography:

Thin Layer Chromatography is a one of the most useful separation techniques available to the organic chemist. Using TLC the chemist can compare reaction mixtures to determine if a reaction has gone to completion, how many chemical constituents are in the reaction mixture (excluding the volatile solvent), and the identity of the constituents themselves. Essentially, the technique relies on the differential solubility of compounds between liquids and solid absorbents. The polarity of every organic compound is different and thus each has unique interactions with solvents. TLC exploits these physical differences. The operating medium is a solid phase absorbent, usually silica, coated on a glass plate. The experimenter uses a capillary device or other transfer mechanism to "spot" a sample of the reaction mixture or pure compound on the surface of the absorbent.

Once the samples are spotted on the plate, the plate is placed vertically in a tank or jar with an appropriate solvent in the bottom. The solvent serves as the mobile phase in the separation technique. To achieve separation, the solute should be somewhat soluble in the solvent mixture. If the solute is insoluble in the solvent, the sample will not move on the plate. When placing the plate in the containing vessel, it is imperative the samples have been spotted above the solvent surface. Otherwise, the samples will simply diffuse off the absorbent into the solvent. Once the plate has been placed in the solvent, capillary action will draw the solvent up the TLC plate. As the solvent moves up the plate, it will interact with the sample. More polar compounds will tend to bond strongly with the polar silica absorbent and thus will not move far up the plate once the solvent begins to migrate up the plate. Less polar compounds tend to move farther up the plate. The location where the solvent stops moving up is called the solvent front.

The distance a spot travels is usually expressed with a normalization term known as the retention factor. The calculation of the retention factor is shown in **Equation 3.**

R_f = Distance traveled by spot / Distance traveled by solvent front

Equation 3

Retention factors can never exceed one. If a substance has a large retention factor, it has a greater affinity for the liquid mobile phase and preferentially dissolves in the migrating solvent. On the other hand, if a substance does not move far, it has a greater affinity for the silica absorbent. Finding the right combination of solvents to achieve separation of compounds is somewhat of a practical art. Oftentimes, the proportions of several solvents must be empirically adjusted until the right balance is found. Another concern is spotting an excess of a sample. This is usually evidenced by "streaking," where a spot exhibits an extended tail on the plate, as highlighted in **Figure 54.**

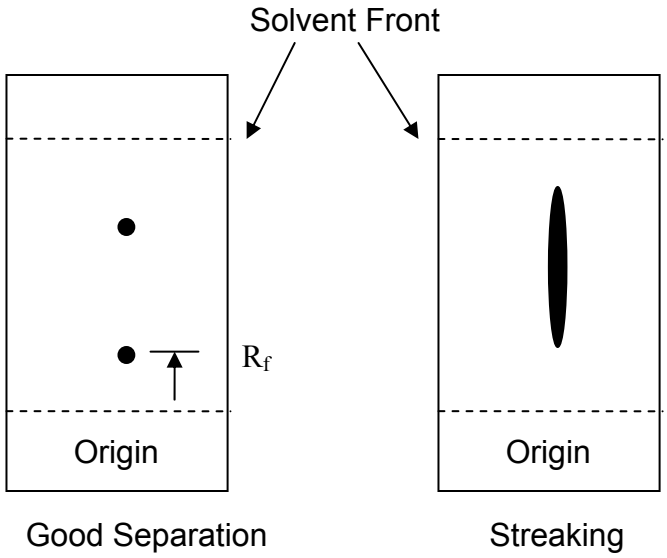


Figure 54: Comparison of Ideal and Poor TLC Spotting

If compounds are invisible to the naked eye, then the spots must be visualized in some way. Some organic compounds absorb ultraviolet light and thus can be imaged by shining a UV lamp on the surface of the plate. However, when compounds do not absorb in the UV range, as is the case in this project, other means must be employed. Iodine chambers, where iodine vapor saturates the plates and spots double bonds, can be occasionally used. The most useful means for our purposes was dipping the plate in a solution of phosphomolybdic acid in ethanol, a green acid that when placed on a hot plate and evaporated, spotted the compounds of interest quite nicely.

With the bicyclic compounds, it has been known that some combination of ethyl acetate and hexane is effective at eluting the compounds appropriate distances up TLC plates. After some investigation, a 3:2 volumetric ratio of ethyl acetate to hexane accomplished the desired migration of both the bicyclic starting compound, the unsaturated ester produced following the first synthetic reaction, and byproducts such as triphenyl phosphine oxide. Usually, 5-ml of this solvent combination (3-ml of ethyl acetate and 2-ml of hexane) would be poured into the containment chamber and allowed to evaporate to saturate the atmosphere with gas-phase solvent.

After spotting a plate with the reaction mixtures or compounds, the plate would be placed in the chamber. Over a period of approximately five minutes, the solvent migrates up the plate. When the solvent front is roughly 10 mm from the top, the plate is removed. Then, the positions are imaged. In the case of the Wittig reaction, the starting compound was spotted on one side and the reaction mixture was spotted on the other. This setup allows the experimenter to determine if the reaction is complete. Once the bicyclic starting spot is gone from the reaction mixture, leaving the product spot only, then the reaction is finished and further purification

procedures can be undertaken to isolate the unsaturated ester in its pure form. Typically, the bicyclic starting compound had a retention factor of approximately 0.35, whereas the unsaturated ester had a retention factor of between 0.50-0.60. This degree of separation allows the spots to be readily distinguished.

II. Flash Chromatography:

Flash chromatography is another useful form of chromatography that extends some of the concepts presented with TLC. In this case, pre-packaged columns of silica were used as a stationary phase. Solvent was then pushed through at a higher rate using increased pressure from a pump. Since it is a closed system, the mobile phase solvent is able to partition through the solid phase much faster than in a simple column procedure. Thus, larger scale separation of compounds can be achieved in a much shorter timeframe than before.

To prepare the mixture for chromatography, several steps were necessary. First, the appropriate column size was determined. Generally, the mass of silica on the column should be 10 times the mass of the mixture to be placed on the column. Then, the necessary solvent combination was prepared and the column was loaded with the solvents. A rule of thumb when separating a compound with a TLC retention factor of 0.50 is to use the solvent mixture that gives a TLC retention factor of 0.35 for the actual large-scale chromatography. Generally, between 400-500 ml of solvent was used with approximately 75-ml being necessary to saturate the silica column. Next, the impure reaction mixture, once the solvent system is evaporated, was dissolved in a minimal volume of dichloromethane. This mixture was loaded onto the column with a Pasteur pipette and the solvent tubing was connected.

Using test tubes, fractions of solvent were collected. The fractions typically range from 2-4-ml of solvent depending on the mass to be separated. Once the entire solvent mixture had been collected in similar fashion, the fractions were tested to determine the location of the sought after compound. Fraction testing was accomplished using TLC. Having already identified TLC solvent conditions for the compound of interest, the fractions were spotted on TLC plates and eluted in chambers with the appropriate solvent mixture. In the case of **compound B**, the unsaturated ester, the conditions were 3:2 ethyl acetate to hexane. The fractions that yielded spots in the same position as the known spot for **compound B** were combined and the solvent evaporated. The result was pure compound to be used in the second synthetic step.

III. Gas Chromatography/Mass Spectrometry:

This technique is actually a combination of an instrument that separates a mixture into its molecular constituents and a device that chemically identifies those constituents. Gas chromatography is the means of separating the mixture. First, the mixture is injected into the column where it is immediately volatized to the gaseous form. The compounds present dissolve into a high-boiling liquid stationary phase. The more volatile components then pass out of the stationary phase and into the mobile phase, which is usually a stream of helium gas. The helium, which is an inert gas, carries the compounds to a detector. The detector plots current against time. The time a compound passes through the column is known as the retention time and is a function of temperature, gas flow rate, and the properties of the stationary phase. As long as these parameters are constant, a particular chemical will produce the same retention time. Thus, retention time can be used as a means of characterization. The area under the peaks is directly

proportional to concentration of the particular compound in the sample injected. An example of a strictly GC plot is given in **Figure 55**, which represents the compound phenyl cinnamate.

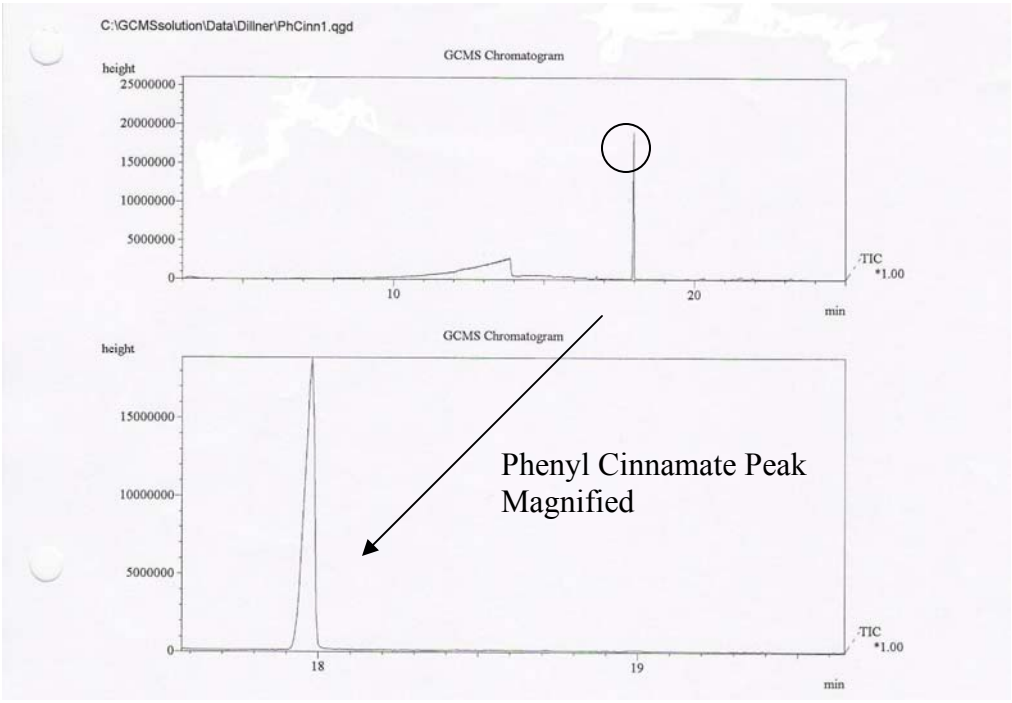


Figure 55: GC Spectrum of Phenyl Cinnamate

Mass spectrometry allows the chemist to determine the molecular weight and structural features of compounds. When combined with gas chromatography, the compounds are actually bombarded with high energy electrons while in the vapor phase. This electron beam ionizes the molecule by knocking out an electron, creating the molecular ion and additional ionized fragments. Fragmentation occurs at the weakest bonds and the locations which will form stable fragments. The resulting ions are passed through a magnetic field, where they are deflected perpendicular to the direction of the field. The amount of deflection depends on the ratio of the mass to charge of the ions. Heavier ions are deflected less than lighter atoms.

Each peak from the gas chromatography portion is given its own distinctive mass spectrometry reading. The mass spectrometry reading from the phenyl cinnamate peak above is shown in **Figure 56.** Because the electrons knock one electron from each fragment, the charges are all +1 and thus the measurement on the x-axis labeled m/z effectively represents the mass of various fragments which result from the splitting. Thus, the molecular ion corresponds to the molecular weight of the compound of interest. Each molecule usually only fragments once; the peak at 224 represents the molecular ion of phenyl cinnamate because it has the same mass as the original compound. The peak at 131 represents the loss of the phenyl group and the oxygen bonded to the carbonyl of the ester. Losing this group is a common fragment for esters.

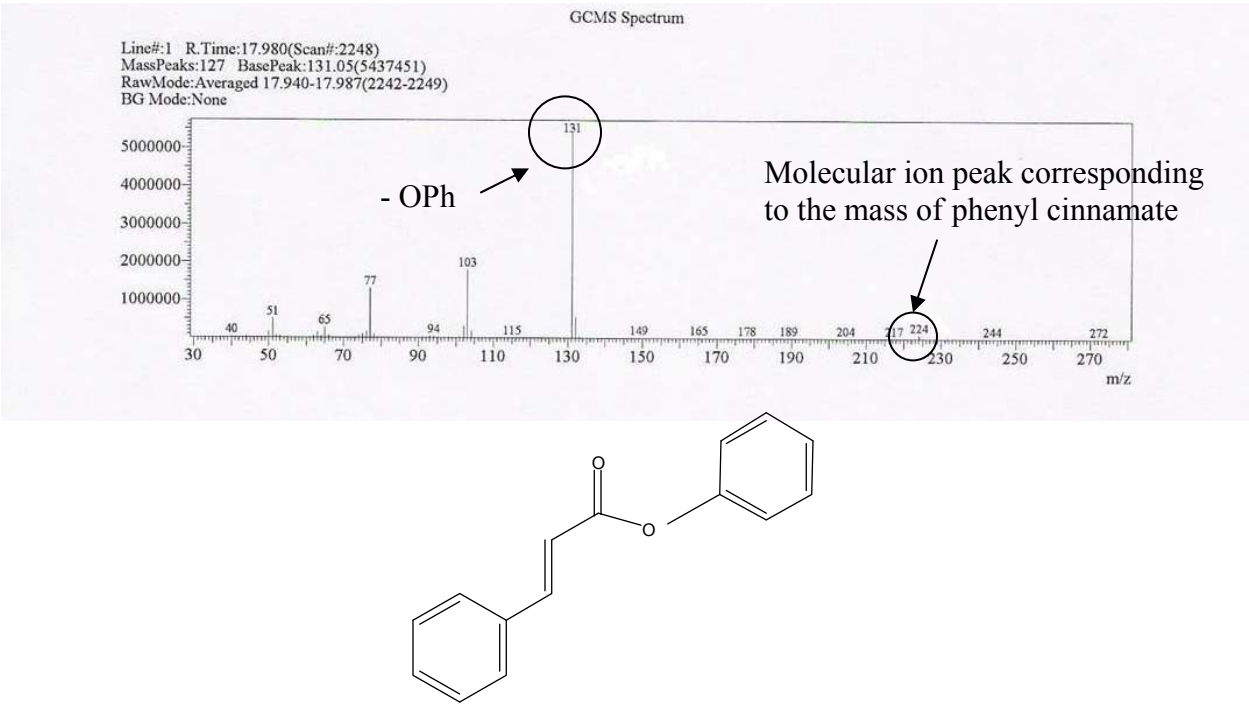


Figure 56: Mass Spectrum and Structure of Phenyl Cinnamate

GC/MS was the technique which proved that conjugate addition could indeed be accomplished using the designed experimental conditions. The pilot reaction of the homocuprate lithium dimethylcuprate adding a methyl group to the number 4 position of phenyl cinnamate was predicted to produce a new compound with a molecular weight of 240 grams/mole. This molecular mass is 16 grams/mole more than phenyl cinnamate and represents the addition of the $-CH_3$ methyl group and hydrogen across the former carbon-carbon double bond. The findings are presented in **Figure 57** shown below. This development was significant, as it demonstrated that the extremely reactive methyl lithium could be kept under argon and not necessarily added in a dry box, which would complicate the procedure.

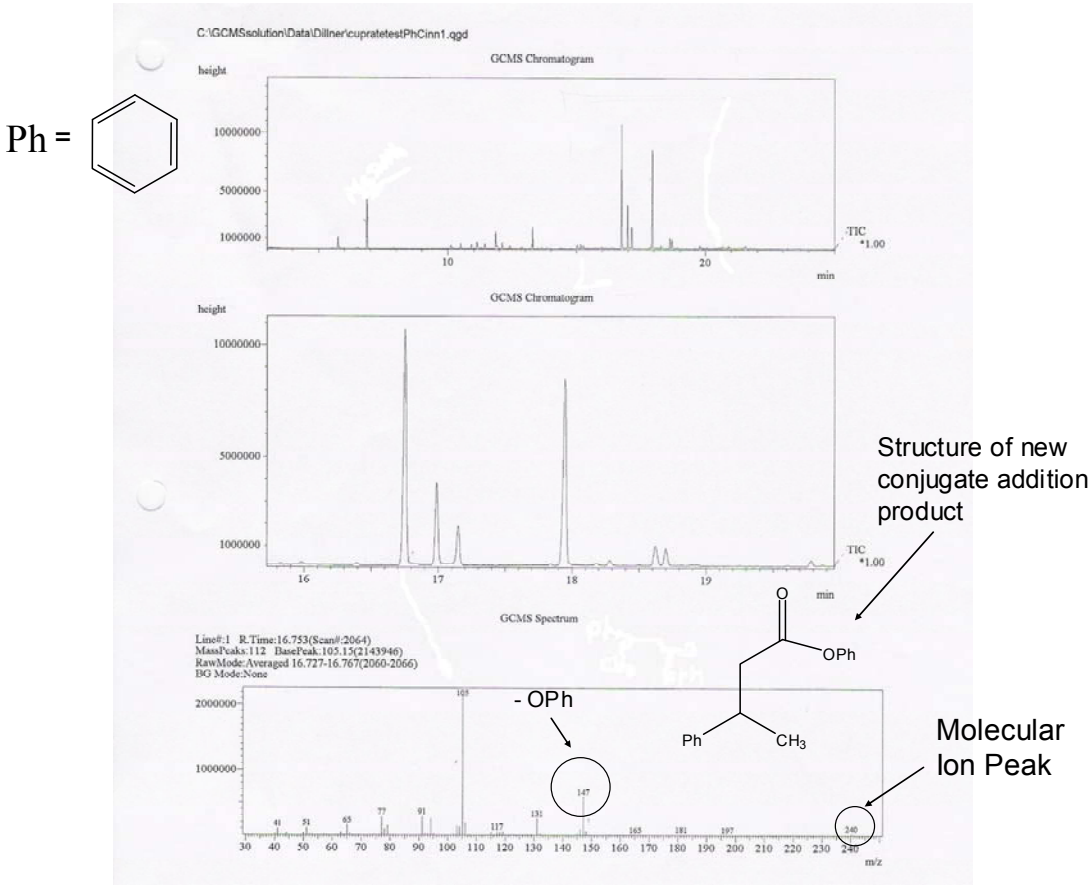


Figure 57: Combined GC and MS for Pilot Conjugate Addition Product

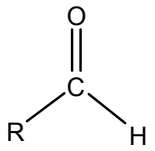
GLOSSARY:

Absorption: taken up within the interior of a substance or within the pores of a solid.

Adsorption: adhering to the surface of a material and not permeating the interior.

Alcohol: any compound that contains a hydroxyl group.

Aldehyde: an organic functional group that consists of a carbonyl group bonded to an organic portion and a terminal hydrogen.



Alkane: an organic compound or portion of a larger compound containing only carbon-carbon single bonds.

Alkene: an unsaturated carbon compound consisting of at least one carbon-carbon double bond.

Alkoxy: an organic functional group that consists of an alkyl group bonded to an oxygen atom, R-O.

Alkyl group: the portion of a compound that has carbons that are only singly bonded to other carbons and hydrogen atoms.

Alkylate: a reaction that results in the addition of an alkyl group to a molecule.

Alpha-carbon (α-carbon): the carbon adjacent to a functional group; usually in reference to the carbon adjacent to a carbonyl group.

Anions: ions with a negative charge.

Aprotic solvent: a solvent that cannot form hydrogen bonds because it is devoid of hydrogens bonded to more electronegative atoms.

Amides: a carbonyl group (C=O) bonded to a nitrogen.

α,β-unsaturated carbonyl: a compound with a double bond conjugated to a carbonyl group.

Beta-carbon (β-carbon): the carbon two positions removed from a functional group; usually in reference to the carbon two positions removed from a carbonyl group.

Bicyclic system: a compound consisting of two fused ring structures.

Biological activity: the effects of pharmaceuticals on living organisms.

Carbanion: a negatively charged carbon atom.

Carbonyl: a CO double bond.

Carboxylic acid: a carbonyl group (C=O) bonded to a hydroxyl group.

Cations: an ion with a positive charge.

Cis orientation: atoms or groups that are on the same spatial face of a molecule; they project in the same direction.

Conformation: a particular shape a molecule can assume through bond rotation.

Conjugate addition: the addition of a nucleophile to the β-carbon of an α,β-unsaturated carbonyl compound; alternatively referred to as 1,4-addition.

Conjugated compound: organic compounds that have one or more *double bond – single bond – double bond* groups.

Degeneracy: having the same energy.

Deprotonate: to remove a hydrogen from a compound.

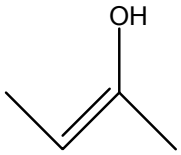
Diterpenoid: a terpenoid formed from four isoprene units; each isoprene has 5 carbons so diterpenoids have 20 carbons.

Dolabellane: class of organic compounds found principally in marine organisms that have demonstrated both antimicrobial activity and tumor killing potential. An archetypical example is shown.

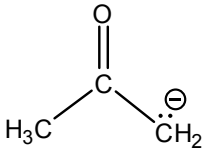
Electrophilic center: electron-deficient centers with partial or actual positive charge.

Enantiomers: non-superimposable mirror image compounds.

Enol: consist of a carbon-carbon double bond with a hydroxyl group bonded to one of the carbons of the alkene.

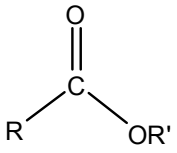


Enolate: anion formed by removing a hydrogen from the carbon α to a carbonyl.



Epimerize: to convert the stereochemistry of a stereocenter to its alternative form.

Ester: an organic functional group that consists of a carbonyl group bonded to an alkoxy group and a carbon structure.



Functionality: groups that bias the reactivity of a compound and dictate its chemical activity and often contain atoms more electronegative than carbon, such as oxygen or nitrogen.

Geminal hydrogens: hydrogens bonded to the same carbon.

Halide: any compound that contains a halogen atom and another species that has less polarity, such as a metal atom or an organic group.

Halogen: elements comprising Group 17 of the periodic table: fluorine, chlorine, bromine, iodine, and astatine.

Heterogeneous catalysts: catalysts that are usually transition metals and do not dissolve in the solvent but rather are suspended in the reaction mixture.

Homocuprate: nucleophilic agents that contain an organic group, lithium, and copper in a respective 2:1:1 ratio.

$$R_2CuLi$$

Homogeneous catalysts: catalysts that dissolve in the solvent and usually contain organic groups that facilitate dissolution bonded to a central transition metal.

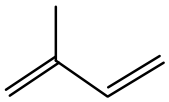
Hydride: a hydrogen anion with a negative charge and a lone pair of electrons.

Hydrogenation: chemical reactions that result in the addition of hydrogen to the carbons of an unsaturated organic compound.

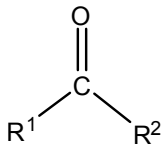
Hydroxyl group: the –OH group bonded to a carbon in an organic molecule.

Isomers: compounds that have identical chemical formulas but different bonding arrangements.

Isoprene: a four carbon chain with conjugated double bonds and a methyl group; the most common hydrocarbon in the human body and a common organic motif in biological systems.

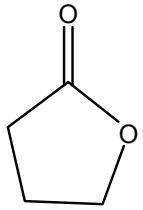


Ketone: an organic functional group that consists of a carbonyl group bonded to two additional carbon atoms.

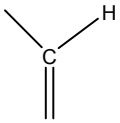


Lactol: a cyclic compound consisting of an alkyl group and a hydroxyl group bonded to the same carbon in the ring.

Lactone: a cyclic ester present in a ring structure derived from the condensation of an alcohol and a carboxylic acid.



Methine: a carbon atom with two single bonds and one double bond, where one of the single bonds is to a hydrogen.



Methyl: the $–CH_3$ group.

Methylene: the $–CH_2$ group.

Nucleophilic centers: electron-rich centers with partial or actual negative charge.

Oxidation: increasing the oxygen content of an organic compound with the net loss of electrons.

Organometallic reagent: a compound with an actual carbon-metal bond.

Phosphine: also known as phosphorus hydride, these groups contain a central phosphorus atom bonded to three hydrogen atoms.

Primary Carbon: a carbon that is bonded to one other carbon.

Protonate: to add a hydrogen to a compound.

Quantitative yield: complete stoichiometric yield; 100% yield.

Quaternary carbon: a carbon that is bonded to four other carbons.

Quenching: to add acid or another chemical that terminates a reaction or modifies intermediates to their final form.

Reduction: lowering the oxygen content of an organic compound or increasing the hydrogen content of a compound.

Reductive elimination: occurs when a central transition metal of a complex loses hydride groups, causing its oxidation state to lower and resulting in effective reduction.

Saturated compound: a compound that does not contain multiple bonds.

Secondary carbon: a carbon that is bonded to two other carbons.

Stereochemistry: chemistry concerned with the three-dimensional spatial configuration of molecules and their bonds.

Stereocenters: atoms where the interchange of two groups produces a stereoisomer.

Stereoisomers: isomers that have the same bonds or connectivity but have a different orientation in three-dimensional space.

Stereoselective reaction: a reaction that favors one isomer over the other.

Stereospecific reaction: a reaction that produces only one of two possible stereoisomers in a reaction.

Steric factors: electronic strain caused by non-bonded atoms close to each other in space or in the molecule which influences a reaction in a predictable way.

Terpene: a compound that occurs naturally in plants and can be considered as resulting from the combination of isoprene units.

Terpenoids: modified terpenes where methyl groups have been added or removed or oxygen atoms added. Most are multi-cyclic structures which differ in both functionality and carbon skeletons.

Tertiary carbon: a carbon that is bonded to three other carbons.

Trans orientation: atoms or groups that are on the opposite spatial face of a molecule; they project in opposite directions.

Unsaturated compound: a compound that contains multiple bonds.

Vicinal hydrogens: hydrogens bonded to adjacent atoms.

Ylide: a negative carbon that is bonded to a positive phosphorus group.

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